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Volume XXIII

Edited by

J. RAMSBOTTOM, B. BARNES and H. WORMALD

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PRESIDENTIAL ADDRESS
LIFE CYCLES OF SMUT FUNGI

By KATHLEEN SAMPSON, M.Sc.

INTRODUCTION

DURING the latter half of the nineteenth century the main features in the life cycles of representatives of the principal genera of the Ustilaginales were made clear by the work of Fischer von Waldheim, Brefeld, Schröter, Winter, Tulasne, Wolff, Kühn, Cornu, Woronin and others. To Brefeld in particular we are indebted for his experimental and descriptive work and for a series of beautiful drawings illustrating the chlamydospores and their mode of germination. On this basis the group was divided into the two families Ustilaginaceae and Tilletiaceae, a system which still prevails with the recent addition of the small family, the Graphiolaceae. This early work is summarized in Plowright's monograph of the *British Uredineae and Ustilagineae* published in 1889 which remains, after nearly forty years, the recognized authority for British smut fungi. When Plowright wrote, cell fusions were known to occur in both families—among the yeast-like sporidia of a smut such as *Ustilago Avenae*, between neighbouring cells in the promycelium of *U. Triticum*, and between the long thread-like cells which crown the promycelium in a *Tilletia* or an *Entyloma*. The behaviour of nuclei in the cells which fused was not observed until later. The time of infection of the host plant had been discovered in several species, and the fact that some smuts are capable of perennating in the host, producing annually a crop of chlamydospores localized in particular organs was clearly understood.

A few years later, in 1893, Dangeard published his classical papers describing in several genera the binucleate condition of the young chlamydospore and the subsequent fusion of the two nuclei. This initiated a long controversy, of which an echo remains to-day, as to the supposed sexual significance of sporidial fusions. The leading protagonists were, on the one side De Bary, on the other Brefeld and Dangeard. As late as 1927, Kharbush concluded a paper on the cytology of certain smuts with the remark that the hypothesis of De Bary rests on an error. I do not propose to treat the subject from this controversial point of view. The data were correct but incomplete, and it was inevitable that some workers should emphasize one fact and some another.

Subsequent research has fully confirmed Dangeard in establishing,

beyond doubt, the fusion of two nuclei in the young chlamydospore when this is formed on the living plant. I have met only one recorded exception, that of *Ustilago Ischaemi* described by Boss (1927), who maintains that in this species both on the host and in culture the smut spores originate in a uninucleate cell. The normal mature smut spore is therefore regarded as a zygote with a single diploid nucleus. It is presumed that on germination a reduction takes place and four haploid nuclei are formed. Two nuclei become associated when sporidial or cell fusions take place soon after meiosis. These events, nuclear fusion, reduction and pairing of nuclei, usually follow in rather quick succession, while the interval between sporidial fusion and nuclear fusion covers the whole of the parasitic phase of development. As we shall see later, our information is extremely scanty concerning the behaviour of nuclei during this important phase of growth.

The fact that several smut fungi can cause serious and sometimes wholesale damage to some important crop plants has stimulated research on a particular and rather narrow range of species. American workers have made valuable and intensive investigations on a few important economic parasites, especially the cereal smuts, but without the stimulating work of Kniep and his pupils in Germany equally interesting but non-economic forms would have remained unexamined. Even so, a large number of species and genera must be investigated before it is safe to generalize about the group as a whole. In present-day literature there is already a tendency to assume for all smut fungi certain facts which have been established for particular species, and I propose to examine the evidence for some of these assumptions. They may be stated rather dogmatically as follows:

(1) Nuclear fusion occurs in the young chlamydospore, and the single nucleus of the mature spore is diploid.

(2) Meiosis occurs at the first or second (perhaps third) division of the chlamydospore nucleus.

(3) Nuclear association is brought about either by hyphal or sporidial fusion with the formation of a dikaryophytic mycelium (that is, the male and female nuclei are paired, not fused).

(4) Gametophytic, that is haploid, mycelium cannot cause infection. Parasitic mycelium is always dikaryophytic.

(5) Saprophytic mycelium is usually haploid. Dikaryophytic mycelium tends in culture to revert to the haploid condition.

CYTOLOGY

In 1899 Harper confirmed Dangeard's statements relative to the fusion of nuclei in the young chlamydospore and studied the behaviour of nuclei in the promycelium. From Harper we have the

figure, now well known in text-books, of *Ustilago Scabiosae*, showing a spore with a four-celled promycelium, each cell of which has produced a uninucleate sporidium, while one nucleus remains in each promycelial cell. Chromosomes and spindles were observed by Harper, but mitosis and meiosis were not distinguished. The chromosome number was given as eight to ten for this species.

Lutman (1910) studied sporidial fusions in several species and traced the passage of the nucleus from one cell to the other. He commented that such nuclei did not appear to fuse, and suggested the possibility of conjugate division. Among the many who have worked on the problem of cell fusions in smuts, only two, Federley (1904) (*U. Tragopogonis pratensis*) and Dastur (1921) (*Tilletia Tritici*) claim to have seen the fusion of nuclei at this stage.

In 1912 Rawitscher published his first paper on the cytology of some smut fungi and showed that the binucleate condition can arise from hyphal fusions (*Ustilago carbo*) as well as from sporidial fusions; his figures of nuclei are among the most convincing yet published. Two years later he illustrated the passage of a nucleus through the H-shaped connexion of two paired sporidia of *Tilletia Tritici* and he affirmed, admittedly without absolute proof, since his wheat failed to head, that successful infection only follows penetration of the host by a binucleate hypha, a fact confirmed more than twenty years later by Flor (1931) and by Hanna (1934).

Rawitscher's work was interrupted by the war, but in 1922 he published an account of the division of the fusion nucleus. The first division occurred either in the spore itself (*T. Tritici*) or in the promycelium (*Cintractia Montagnei*). The spindle was intranuclear, chromosomes were formed and Rawitscher suggested, rather tentatively, that the haploid number is two. He found that hyphal fusion took place in *C. Montagnei* by long or short bridges between promycelial cells, which explained the somewhat unusual figures by Brefeld, who described in this species a two-celled promycelium with two sporidia.

In the early part of the present century, the germination of spores and the cell fusions which follow were studied in a wide range of species, and it became clear that the elements which unite have a variety of form, such as the small yeast-like sporidia of *Ustilago Avenae*, the cells of a promycelium which form either a long bridge or a buckle joint, the more elongated or filamentous sporidia in the Tilletiaceae (*Urocystis Anemones*), which can fuse at the tip, the middle or the base. While the mode of fusion is on the whole characteristic of the species, it is not necessarily constant under all conditions. Hüttig (1931) discovered, for example, that in several species of *Ustilago* the type of germination, and consequently the type of fusion, can be controlled to some extent by temperature. Stakman *et al.*

(1934) found that the binucleate condition could arise in several different ways in the one species, *Urocystis occulta*. Some promycelia possessed only two sporidia, but these were binucleate and presumably the nuclei paired in such cases before leaving the promycelium. Some species for long remained puzzling by their lack of fusions. Notable examples are *Ustilago Zeae*, *Doassansia Sagittariae* (though fusions occur readily in the closely related *D. Alismatis*) and *Urocystis Cepulae*. I shall return to this point in a later section.

Since 1922, work on smut fungi has tended more and more in the experimental direction and only a few workers have attempted to deal with cytology. Kharbush (1927, 1928), and Wang (1932*a, b*; 1934), working in Dangeard's laboratory, studied the division of nuclei in germinating spores. They agree in supposing that the haploid number of chromosomes is two in all species studied. Dickinson (1931) found more than two in *Ustilago Kolleri* but gives no illustrations. Wang, who describes the cell in detail, states that the first division is meiotic. Her figures support this in some degree, but I doubt if we can regard meiosis as absolutely established from cytological evidence alone. By analogy with other fungi, this is the stage at which a reduction division is expected and the data which I have summarized under the heading "Experimental Evidence" seem to confirm this view, but it would be unwise to become dazzled by the fascinations of smut genetics and to forget how few are the established facts concerning chromosome behaviour in this group of fungi. To obtain a convincing cytological picture of meiosis in this group is no easy task. The nuclei of smuts, even in the resting spore, are extremely minute, something like 3μ in diameter, that is about the size of the nucleolus of the nucleus in *Vicia Faba* and less than quarter the size of the nucleus in the cells of the root tip. Added to this is the difficulty of getting a large supply of germinating spores conveniently fixed to the slide. Cytological investigations have been attempted, however, in a few species and we can still hope that material may be found which will yield to the technique of fungal cytology.

EXPERIMENTAL EVIDENCE THAT MEIOSIS OCCURS ON THE GERMINATION OF THE CHLAMYDOSPORE

During the past fifteen years abundant data have accumulated dealing with the genetics of smut fungi, based on the assumption that meiosis occurs when the chlamydospore germinates. These studies were inspired by the work of Kniep (1919) on *Ustilago violacea*. By preparing mono-sporidial cultures and mixing them in pairs, Kniep was able to show that they could be arranged in two groups. Fusion occurred between members of opposite groups and

failed if cultures from the same group were mated. In his book on the sexuality of lower plants (1928) Kniep names fourteen species of *Ustilago* in which he or his pupil Bauch found this simple type of heterothallism. Dickinson (1927-8) added *U. Kolleri* to this list.

Work of this kind necessitates delicate micro-manipulation, one of the outstanding advances in technique of the present century. Kniep (1926) found in a wide range of species of *Ustilago*, that fusion would take place between appropriate sporidia of different reticulate-spored species. He was able also to cross species within the group with smooth or echinulate spores, but he failed to get fusions between these smuts and those having reticulate markings on the spore. Cell fusions only are in question here, as there is no evidence that a nuclear fusion follows in these so-called interspecific crosses.

Kniep (1928) uses the term gametes for the units which fuse, noting that there is complete morphological isogamy, for not even a difference in size could be established between the *a* and *b* sporidia. In some species, however, a physiological difference appears to exist, for example in *U. violacea*, f.sp. *Dianthus deltoides*, since *b* colonies are suppressed on certain media, notably on malt agar, while *a* colonies flourish (Bauch, 1922, 1927) on that medium. In mixed plates this leads to the elimination of one type of gamete and consequent failure to get fusions among the sporidia which remain or develop in sub-cultures.

More recently, Thren (1937) obtained haploid cultures of *Ustilago nuda* by germinating the spores first at room temperature and then keeping them at 2° C., this low temperature having an effect on the promycelium which made it possible to separate the cells with a micro-manipulator (see also Lange de la Camp, 1936). Thren found that + haplonts could be distinguished from - cultures by their stronger growth. The correlation was not quite perfect, 114 cultures of strong growth were all + while among 135 cultures of weak growth, 133 were - and two were +. On malt agar both types flourished, but on potato agar only the - type grew, a result similar to but not identical with that obtained with *U. violacea*.

Experimental investigations have followed somewhat closely the parallel work in the Hymenomycetes which began a few years earlier. Thus the species which gave more complicated results than those mentioned above are interpreted as tetrapolar, fusion being controlled by two pairs of allelomorphic genes, as in *U. longissima*. Bauch (1923) and Kämmerling (1929) described this fungus as having three sex groups, an arrangement not readily explained on Mendelian principles. Later, Bauch (1930) discovered two types of fusion and suggested that failure to distinguish them had complicated his results. Type 1 is the normal fusion between gametophytes differing in two factors; type 2, which he calls a tangle fusion, occurs

between gametophytes differing in only one sex factor. A somewhat similar hypothesis based on the development of false clamps (that is, clamps which do not actually fuse with the parent hypha) has been put forward to explain the illegitimate pairings in certain Hymenomycetes.

There are diverse opinions as to what these factors shall be called. Some use the term sex factors for both genes, some suppose that the first represents a sex factor and the second a sterility factor, while others prefer to regard both as sterility factors. Quintanilha (1935) discusses these points of view in a recent paper dealing with *Coprinus fimetarius*. The term copulation factor which is also in use has the merit of being non-committal.

Bauch's work on *Ustilago longissima* var. *macrospora* is interesting from another standpoint. This is one of the species which Brefeld, on account of the method of germination, placed in his pro-group of smuts, since it does not cut off lateral sporidia in a regular manner from a four-celled promycelium (*U. Scabiosae* and *U. violacea* type). Instead, the chlamydospore puts out on germination a short pointed process which cuts off sporidia terminally and in succession. Bauch discovered, by staining and experimental technique, that the first sporidium receives two nuclei which are usually of different sex. When this sporidium becomes septate and multiplies by further budding, uninucleate sporidia are formed which can fuse among themselves. Later sporidia cut off from the original germ tube are uninucleate, and their descendants, in contrast to those of the first sporidium, will not fuse *inter se* (Bauch, 1930).

Dickinson (1928, 1931), working with *U. Kollerii*, was able by isolating the individual sporidia from the four segments of a promycelium to study the arrangements of *a* and *b* sporidia on the promycelia arising from different spores. He found that segregation for sex factors can take place at either of the first two divisions of the resting spore nucleus. This has been confirmed by Holton (1931) and by Popp & Hanna (1935). Dickinson also examined the rather undefinable cultural characters such as colour, marginal growth, surface indentations and elevations, and concluded that segregation for such factors might be delayed until the third or fourth division of the fusion nucleus. Similar results were obtained by Holton (1931, 1932) working with the oat smuts and by Christensen (1931) for *U. Zeae*, but Western (1936) found no evidence of delayed segregation in his material of *U. Avenae*. Surely all those who love law and order must hope that another explanation will be found. Already we have to admit that fungi are far too much addicted to mutation, but to have them also procrastinating at meiosis savours too much of anarchy. I was asked, when discussing with a cytologist the possible mechanism of delayed segregation, if smuts had many chromosomes

or if they were tetraploids. The fact that we are dealing with an organism reputed to have only two chromosomes adds to the difficulty of understanding how segregation can spread over several nuclear divisions, but perhaps we are too anxious to press the fungi into the framework of modern cytological theory.

Returning to the more straightforward genetical data, reference must be made to the extremely interesting results arising out of the careful and detailed work on some of the species of economic importance in the U.S.A. A definite step forward was taken by those investigators who made observations on several successive chlamydospore generations. I shall only have time to deal with some of their conclusions. Allison (1935, 1937), working with barley (*U. Hordei* $\times$ *U. medians*), and Holton (1936*a*) with oats (*U. Avenae* $\times$ *U. Kollerii*), found echinulation dominant over smoothness with a simple 3 : 1 segregation. Popp & Hanna (1935) confirmed this in the oat smuts. Holton (1936*b*) worked also with a buff type of smut, a form having smooth hyaline spores which he thinks originated as a mutant from *U. Kollerii*. In crosses with the latter species, brown was dominant to hyaline and gave a simple segregation.

The two common oat smuts *U. Avenae* and *U. Kollerii* were found by Holton and others to be completely interfertile, but sporidia arising from the F_1 chlamydospores are largely non-viable. It is suggested that this apparent lack of fertility in hybrid material is a reason for regarding the parents as distinct species, but, as Holton (1932) points out, sterility is not universally accepted as an adequate criterion for separating species.

Heterothallism of a complex type was first discovered in *Tilletia* by Flor (1931, 1932) who also successfully crossed the two species *T. Tritici* and *T. levis*, and obtained hybrid spores with smooth walls.

Later Hanna (1934), working apparently with single physiologic races of *T. Tritici* and *T. levis*, found heterothallism of the monohybrid type, smoothness again dominant over reticulate walls, and the presence of trimethylamine which gives the fishy smell to *T. Tritici* bunt likewise behaved as a dominant. Becker (1936) also found only two sex groups in the monosporidial lines arising from a single chlamydospore, but haploid lines of diverse origin gave a more complex result. This is taken as an indication of the existence of geographic races such as we have in some of the Hymenomycetes and in *Diaporthe perniciosa* investigated by Miss Cayley (1931).

Similar studies have been made on certain sorghum smuts, notably by Rodenhiser (1932). *Sphacelotheca Sorghi* and *S. cruenta*, two heterothallic bipolar species, cross readily but give evidence of genetic sterility in the hybrid. Echinulation was found to be dominant and a back-cross to the recessive parent gave 1 : 1 segregation.

Tyler & Shumway (1935) crossed *Sphacelotheca Sorghi* with *Soro-*

sporium Reilianum and found the F_1 intermediate in spore and sorus characters. Vaheeduddin (1936) obtained a fertile hybrid between *S. Reilianum* and *Sphacelotheca cruenta*. He noted that the F_1 promycelia and sporidia were larger than those of either parent, and suggests that this is due to heterosis. Goldschmidt (1928) in his crosses between physiologic races of *Ustilago violacea* also noted that the promycelia arising from hybrid chlamydospores were considerably longer than those of either parent. He doubts if this can be hybrid vigour since the promycelium is a series of haploid cells, not a sporophyte. As, however, the growth in length of the germ tube issuing from a spore is probably controlled by the nucleus of that spore, which is the only true sporophytic nucleus in the life cycle of a smut, Goldschmidt's argument against heterosis does not in my opinion hold. At this stage of the work it is natural that the behaviour of fungi should be interpreted by reference to genetical theory based on the higher plants, and it is not surprising to learn that a lethal factor has already been recorded in one of the smuts. Chilton (1938) found this abnormality in certain haploid lines of *U. Zeae*, the presence of the factor causing lysis of the promycelium.

The much investigated maize smut, *U. Zeae*, has been left until the end, since it is the most puzzling species so far studied. To mention only a few of its idiosyncrasies: *U. Zeae* produces sporidia which do not fuse readily,* its characters when studied genetically invariably show a multiple-factor type of inheritance, it is especially subject to mutation, and it is alleged to have put off segregation through one if not two chlamydospore generations. For this intimate knowledge of a difficult species we are indebted chiefly to Prof. Stakman and the many pupils whom he has inspired to study fungi (Stakman *et al.* 1927-35; Eddins, 1929; Moore, 1932; Christensen, 1929, 1931; also Sleumer, 1932).

It is not possible to deal with these elaborate studies in detail, but I must refer to a feature which seems at present to be peculiar to *U. Zeae*, namely, the existence of the so-called solo-pathogenic lines. *U. Zeae* is described as "predominantly heterothallic", that is to say, normally two lines of opposite sex are required for successful infection, but a small percentage of monosporidial lines will invade the plant and produce a crop of chlamydospores. Christensen (1931), who made a most intensive study of this problem, found this unusual behaviour in the monosporidial cultures arising from seven chlamydospores in a total of 275 which he examined. If this were straightforward parthenogenetic development, the resulting smut spores should be either haploid and of one sex or, if doubling of the chromosomes occurred, a condition which not infrequently accompanies

* Sleumer (1932) has been most successful in observing the fusion of sporidia in *U. Zeae*, transferring them from young cultures to weak alkaline water (pH 8-8.3).

parthenogenesis, they should be homozygous diploid. What actually happens is that the solo-pathogenic lines yield chlamydospores which segregate for sex on germination. It is supposed that segregation was incomplete in the parental chlamydospore, and the solo-pathogenic lines are described as being uninucleate but "diploid at least for certain characters". In culture, solo-pathogenic lines are as stable as unisexual lines. Moreover, the passage of a solo-pathogenic line through the living host does not change its cultural characteristics nor its pathogenicity when the line is reisolated before chlamydospore formation. The following is an extract from a letter from Dr Christensen in which he was kind enough to answer the question why he considers that the behaviour of solo-pathogenic lines is due to failure of reduction rather than mutation: "I believe that the solo-pathogenic lines are diploid due to the failure of reduction, rather than mutation, because of the following: (1) the monosporidial progeny from a single chlamydospore are usually all solo-pathogenic; (2) chlamydospores produced by these solo-pathogenic lines may give rise to several different sexual groups; (3) many hundred monosporidial lines have been studied in our laboratory, but there has never been a mutation involving a sexual reversion (+ to - or vice versa). Moreover, none of our unisexual monosporidial cultures have ever changed to solo-pathogenic lines while under observation."

The maize smut should provide interesting material for the cytologist when the less abnormal smuts have been adequately investigated.

In addition to the more easily defined and clear-cut characters such as spore markings and colour which have been discussed, cultural characteristics and pathogenicity appear also to obey the accepted laws of Mendelian segregation (Dickinson, 1928; Isenbeck, 1935). Goldschmidt (1928) suggests from his work with *U. violacea* that the two physiologic races on *Silene Saxifraga* and *Melandrium album* differ by only one factor. He was able to infect both hosts with his hybrid material and concluded that it is only necessary in *Ustilago violacea* to have one nucleus specialized for the host plant, which means, I take it, that in this particular cross pathogenic behaviour shows blended inheritance.

It is not the purpose of this paper to give an exhaustive account of the experimental work upon which the genetics of smut fungi rest. Sufficient has been said to show that a rather elaborate structure of genetical theory has been built during the past twenty years. Absolutely essential to this theory is the supposition that the two nuclei which fuse in the chlamydospore are the direct descendants of those nuclei which became associated when copulation occurred between appropriate gametic elements. Many cell generations bridge the interval between these two crucial points in the life cycle of a

smut. On analogy with other groups of fungi we look for some form of conjugate division to ensure the meeting of two nuclei of opposite sex in the chlamydospore initial. Unfortunately information about the behaviour of nuclei in the parasitic mycelium of smuts is very incomplete. The close intertwining of mycelium with the cells of the host plant makes the examination of individual hyphal cells extremely difficult and again one must plead the small size of the smut nuclei. Some of those who have stained for nuclei, state that the cells of the parasitic mycelium are uninucleate, others that they are multinucleate or that both types occur in the same species; few have observed any tendency for the nuclei to remain associated in pairs. We may take it, I think, that parallel conjugate division of the rust type is certainly not a regular feature of smut mycelium.

PARASITIC MYCELIUM OF SMUT FUNGI

Seyfert (1927) alone has undertaken a serious search for conjugate division in the parasitic mycelium of the Ustilaginales. He studied first a smut which attacks *Luzula*, *Ustilago Vuijckii*, a species in which clamp mycelium was recorded as far back as 1895. It seems that clamps are such a regular and even striking feature of this mycelium, especially between the young chlamydospore cells, that Liro (1924) figured and described them as antheridia which were supposed to fertilize the oogonia, that is, the chlamydospore initials. In this species Seyfert studied the development of a clamp and the behaviour of associated nuclei and found it comparable with the process of clamp formation in the Hymenomycetes. Seyfert then looked for clamps in the parasitic mycelium of a number of species of *Ustilago* as well as in members of the genera *Doassansia*, *Urocystis*, *Tilletia*, *Entyloma* and *Tubercinia*, and found them in all. He notes, however, that clamps were rare and not quite normal in *Tubercinia primulicola*. Seyfert points out that microtome sections are useless in the search for clamps as they do not give a sufficient length of mycelium. His method was to scrape the particular parts of a plant in which spores would eventually be formed; consequently his observations do not furnish information about the early stages of parasitic growth. A point which presents some difficulty to me is that Seyfert's figures closely resemble some of those used by Harder (1927) to illustrate false clamps in the Hymenomycetes. Christensen states from his observations of clamps in *Ustilago Zeae*, that the clamp-like tube did not appear to fuse with the hyphae, and Sleumer (1932) failed to find structures which he could accept as true clamps, though incipient branches superficially resembling clamps were seen. It would be interesting if we could get more information concerning the actual process of development of clamp connexions in the

Ustilaginales and they should certainly be recorded if found in any new species. Kaiser (1936) notes the absence of clamps in *Entyloma Arnoseridis*, and Kolk (1931) did not describe them in *Ustilago Avenae*. We must, however, avoid overestimating the significance of clamps, since it is known that in the Hymenomycetes they are not invariably associated with conjugate division. Vandendries (1937) quotes *Collybia tenacella* as an example of a heterothallic species without clamps, and in the homothallic species *Calocera cornea* clamps are also absent.

The imperfect state of our knowledge concerning nuclear behaviour during the parasitic growth of the smut fungi is partly responsible for the persistence of the view that sporidial fusions have no sexual significance, but those who still hold this opinion do not offer an alternative explanation of the facts which suggest compliance with Mendelian laws.

It will be convenient now to return to our fourth postulate, viz. that parasitic mycelium is always dikaryophytic. Responsibility for this view rests with those who, working with species like *Ustilago Avenae* having clear sporidial fusions, found that monosporidial cultures failed to cause infection. At what point is the dikaryophytic phase initiated in species which lack sporidial fusion? *U. Zeae* was for a long time regarded as an exception, since Rawitscher (1912) maintained that mycelial cells of this species were uninucleate in the host until the wall dissolved between adjacent cells just prior to the development of chlamydospores. Seyfert (1927) found clamp mycelium at an earlier stage than this, and Hanna (1929) has shown that fusion of fine haploid mycelial strands may occur immediately after penetration of the host. It does seem that *U. Zeae* may differ from the other smuts which attack cereals in that haploid mycelium can at least penetrate the host, but we do not know how long it will persist. From Sleumer's work on this species it seems that the cells of the parasitic mycelium are binucleate in the early stages of invasion but later uninucleate as Rawitscher claimed.

As I have already indicated, the time and manner of fusion differs in closely related species. Thus in *Doassansia Alismatis*, sporidia fuse in pairs as in *Tilletia Tritici*, while this is absent or very rare in *Doassansia Sagittariae*, which seems to present a similar mode of germination. Fisch (1884) figured one fusion but commented that fusion is very rare and that invasion of the host results from one sporidium only. It is not yet known at what point the binucleate condition arises in *D. Sagittariae*, but it is one of the forms in which Seyfert found clamp mycelium.

A somewhat peculiar condition was described by Blizzard (1926) in *Urocystis Cepulae*, the onion smut, and, as far as I know, it has not been reinvestigated. He found no fusion among sporidia or between

cells of the saprophytic mycelium. Infection arose from the uninucleate cells of the saprophytic mycelium, and the parasitic mycelium was also uninucleate for some time, becoming binucleate just before sporulation. Blizzard assumed, therefore, that this fungus had a long haploid phase, and if the facts are correct, *U. Cepulae* may provide the first example of a homothallic species of smut. *Graphiola Phoenicis* provides a parallel, since, according to Killian (1924), the cells of the parasitic mycelium and the young fructification are haploid. The dikaryophytic phase is relatively short and does not owe its origin to cell fusions.

Ustilago Ischaemi is of interest as the only species reputed (Boss, 1927) to form chlamydospores from a uninucleate cell. Kniep (1928) sees in this a parallel to those Hymenomycetes (such as *Hygrophorus conicus*) which pass their whole development in the haplo-phase. Actually we do not know if the nuclei of this smut are haploid, and it might conceivably be one which develops sporophytic instead of dikaryophytic mycelium, though by analogy the haploid hypothesis is probably correct.

There may be other exceptions, and it is important to remember that only a comparatively small number of species of smuts has been examined in any detail, but the evidence now available leads us to expect in parasitic smut mycelium two types of nuclei associated in some way or other. How permanent such an association may be will be discussed later.

The fifth postulate concerns saprophytic mycelium.

GROWTH OF SMUT FUNGI IN CULTURE

Smut fungi have been grown in culture since the time of Brefeld (1883) who obtained in his cultures of *Tilletia Tritici* cells which closely resembled chlamydospores. Smuts in culture are not picturesque, often forming on solid nutrient media a grey-brown gelatinous looking mass, sometimes with a strongly corrugated surface resembling miniature mountains and river valleys, so that descriptions refer to the topography of a culture. These rather undefinable characteristics have been used in genetical studies with some quite clear-cut results, and I admit that one can learn to recognize the face of a colony with the accuracy of a farmer who knows his own sheep. What is more difficult is to write a description by which someone else will recognize the same features. Our immediate concern is the development of chlamydospores which takes place at times in old cultures. It has been recorded for the following species: *Tilletia Tritici* (Brefeld, 1883; Sartoris, 1924; Buller, 1933), *Ustilago Zeae* (Grüss, 1902; Sartoris, 1924), *U. Tritici* (Sartoris, 1924; Schaffnit, 1926; Rodenhiser, 1928), *U. Hordei* (Fleroff, 1923;

Sartoris, 1924; Rump, 1926; Wang, 1932a), *U. nuda* (Rodenhiser, 1928), *U. Avenae* (Fleroff, 1923), *U. Ischaemi* (Boss, 1927), *U. Heuffleri* (Sartoris, 1924), *Urocystis Anemones* (Kniep, 1921), *Entyloma Ranunculi*, *E. Calendulae* (Stempell, 1935), and *Sorosporium Reilianum* (Potter, 1914). In the cereal smuts chlamydospores have been obtained repeatedly by different workers. Unfortunately few of those who have seen them in culture have obtained information about their nuclei or mode of germination. Sartoris (1924) obtained chlamydospores of five species and in *Ustilago Heuffleri*, a species which attacks the dogtooth violet, he records that there were two nuclei in the young spore, that these fused and that the mature spore germinated to give a typical four-celled promycelium with lateral sporidia. Surely here at least the normal life-cycle of a smut has been completed in culture.

Fleroff (1923), working with *Ustilago Avenae*, states that one race produced in culture chlamydospores which germinated to give a promycelium with clamp connexions, but no mention is made of nuclear behaviour. Stempell, whose work on *Entyloma* will be mentioned again, obtained in culture both haploid and diploid spores of *E. Calendulae*, the former germinating by a simple germ tube which formed a long hypha and the latter by a promycelium with terminal sporidia characteristic of the genus. *E. Ranunculi* on the other hand produced only haploid chlamydospores in culture.

Dickinson, working with oat and barley smuts, found that dikaryophytic mycelium soon reverted to the haploid type, but it seems that this does not happen in all species, since two at least (*Ustilago Heuffleri* and *Entyloma Calendulae*) can complete their life cycle on artificial media in a manner seemingly equivalent to that on the host plant. Thren (1937), whose work on *Ustilago nuda* has already been mentioned, found that he could distinguish the cultural characteristics of haplonts and dikaryonts and he comments on the stability of the latter. Segregation did occur, but only sporadically, and the haploid mycelium could be recognized, the effect of the two types growing together resembling a colony with saltants.

It seems, therefore, that smut fungi adopt rather readily the saprophytic life and that both haploid and dikaryophytic mycelia can grow in culture. The degree of permanence of the latter type apparently varies with the species.

SPORE PRODUCTION IN SMUT FUNGI

To understand the true homology of the various units we call spores in fungi, and to get a reasonable system of nomenclature is not a simple matter, as Vuillemin (1910-11) and Mason (1937) have shown in their studies on the *Fungi imperfecti*.

The chlamydospores, or, as they have been called, teleutospores,

upon which the classification of smut genera rests, are diploid, equivalent to zygotes, and they alone represent the true sporophyte generation.

In the Ustilaginaceae, the small cells which are cut off from the promycelium and increase almost indefinitely by budding under suitable conditions, are usually termed sporidia. Normally they are haploid and provide for the multiplication of the gametophytes and their nuclei function as gametes. In *Ustilago longissima* and possibly in other species sporidia containing nuclei of both sexes may be formed (Bauch, 1923).

In the Tilletiaceae, crescent-shaped spores (the so-called primary and secondary conidia) are produced either before or after fusion of the long needle-shaped promycelial spores which were formerly called sporidia and regarded as homologous with basidiospores. Buller & Vanterpool consider that since the crescent-shaped conidia are eccentric and are discharged by a water-drop mechanism, these and not the promycelial spores are equivalent to basidiospores (Buller & Vanterpool, 1925; Buller, 1933; Vanterpool, 1932).

It appears from the work of Dastur (1921), Boss (1927) and Hanna (1934) that the primary conidia are binucleate, while the secondary conidia are uninucleate and presumably haploid. Only low infection resulted from secondary conidia, although originating in mycelia of opposite sex, while primary conidia produced nearly 100% infection (Hanna, 1934).

Hanna (1929) noticed that maize leaves floating on sugar solution produced minute uninucleate cells in aerial chains which broke up into units described as conidia. If our conception of the parasitic mycelium is correct this must provide an example of the origin of haploid conidia from dikaryophytic mycelium. I do not think it was ascertained if these conidia were of two sexes.*

Uninucleate aerial conidia, looking in the figure like microconidia, were described by Boss (1927) as covering his cultures of *Ustilago hypodytes*. Aerial conidia borne in long chains or in clusters are produced on the surface of culture media by *Tolyposporium filiferum* Busse (Busse, 1904; Kamat, 1933). *U. Vujičkii* also is interesting. The young capsules of *Luzula* infected by this species are described by Seyfert (1927) as full of a white doughy mass which consists of uninucleate spores. Unfortunately it is not clear what part they play in the dissemination or life cycle of the fungus. Reukauf (1906) refers to somewhat similar conidia in the leaves of frogbit attacked by *Tracya Hydrocharidis*, but their connexion with the smut was not proved.

* In the Hymenomycetes oidia are a characteristic feature of haploid mycelia. Haploid oidia are sometimes cut off from diploid mycelium (Gilmore, 1926) and more rarely diploid oidia are produced (Hanna, 1938). See also Martens and Vandendries, 1933.

Two genera, *Tuburcinia* and *Entyloma*, are of special interest in this connexion, for they have been known since 1883 to possess conidial stages. Conidia were recorded but not figured by Setchell (1892) in *Doassansia Martianoffiana*.

Tuburcinia, doubtless because of its localized distribution, has been little investigated. Malcolm Wilson (1915) gave a short account at a British Association meeting of *T. primulicola* on *Primula vulgaris*. He found that the perennial mycelium was septate and consisted of uninucleate cells. In the young flower the stamens were glued together by masses of small uninucleate conidia which were said to conjugate in pairs. A few weeks later chlamydospores were produced in the floral organs. They were binucleate when young and germinated to give sporidia, but their fusion was not observed. The implication is that in this species the first phase of parasitic growth is haploid and the later phase is dikaryophytic. This species would make an interesting subject of study by the modern technique of monosporidial cultures, since it is by no means clear if the conidia are responsible for the change from one phase to the other. If the conidia are homologous with the crescent-shaped spores of *Tilletia Tritici* it would be interesting to know if they are produced in culture and if they show the same method of separation from the sporophore.

Entyloma is a less uncommon genus and it is represented by a fairly large number of species, but not all are known to possess a conidial stage. Among the nine British species only four are known to have conidia. In the absence of chlamydospores the conidial stage is identified either as a *Ramularia* or a *Cylindrosporium*, two form genera which grade into one another. Marchal & Sternon (1925) state that only the presence of chlamydospores enables one to decide whether any given form is a Hyphomycete or a smut. This being so it is difficult to use the generic name *Entylomella* which von Höhnelt (1924) proposed for the imperfect stage of *Entyloma*.

There are in Britain two species of *Entyloma* on the genus *Ranunculus*, *Entyloma microsporum* and *E. Ranunculi*. The latter, which produces white flecks on the leaves of the lesser celandine in spring, was described and beautifully figured by Marshall Ward (1887). He showed that the sickle-shaped conidia arose from parasitic mycelium before chlamydospores are formed, and therefore that they could not be connected with the germination of smut spores, which are produced later. Fusions between conidia and penetration of the host plant, were studied and illustrated. A statement that "fusion does not affect the fate of the conidia" seems to imply that Marshall Ward thought invasion of the plant could arise from either solitary or fused conidia.

The same species and *E. Calendulae* have been investigated from another angle by Stempell (1935). The sickle-shaped conidia are said

to be haploid in view of their single nucleus. They give rise in artificial culture to a haploid mycelium which forms the same type of conidium again, and these are said to be discharged by the water-drop mechanism as in *Tilletia Tritici*. Stempell failed to obtain fusions between these conidia or the mycelia which they produced. Haploid chlamydospores developed in culture after some weeks.

Stempell was able to grow the dikaryophytic mycelium of *Entyloma Calendulae* in culture and this produced another type of conidium which he described as half-moon-shaped and binucleate; in a table of homologies he draws attention to the fact that these correspond to the uredospores of rusts.

Binucleate conidia were also found in culture by Kaiser (1936) working with *E. Arnoseridis*, a species closely related to *E. Calendulae*, but these were long and needle-shaped, quite distinct in appearance from those of *E. Calendulae*. Kaiser also investigated the species of *Entyloma* on Boraginaceae, and on *Myosotis palustris* he found two distinct types of conidia occupying the same leaf, a needle-shaped form on the upper surface and an ellipsoidal type on the lower side of the leaf. Both types are said to have two or more nuclei.

Species of *Entyloma* clearly present some interesting problems, and so far no species has been investigated by the technique of infecting plants with monospore and paired cultures. The role of conidia in the two genera which possess them is not clear, and it is premature to assume that they function like the spermatia of rusts. If penetration can succeed with or without fusion, as Marshall Ward implied, it seems that the same tissue of a host might contain both gametophytic and dikaryophytic mycelium. A survey of the group shows that we cannot assume that the parasitic mycelium of all smut fungi is invariably dikaryophytic, though experimental data lead to the conclusion that this is true in some species. Both haplonts and dikaryonts can be grown saprophytically, but the latter show differing degrees of instability. The partnership of the two nuclei which form the synkaryon is capable of dissolution both in culture and on the host plant, a feature which links the smuts with the Hymenomycetes rather than the Uredinales. In rusts, the condition of paired nuclei once it has arisen, is, so far as we know at present, rigidly maintained. Perhaps something of the same unshakable loyalty attaches the rusts to their host plants since their obligate parasitism remains unbroken. Comparing the three groups from the present angle of discussion, the relative plasticity of the Hymenomycetes and the Ustilaginales seems to draw them together in contrast to the more highly specialized Uredinales.

BIOLOGIC SPECIALIZATION

It would surely be improper for a plant pathologist to conclude with anything so unpractical as an evolutionary tendency, and I propose to add a few words on the problem of biologic specialization which has been implicit but not previously discussed in this survey. Since the work of Reed (1920) on the oat smuts and that of Zillig (1921) on anther smut of the Caryophyllaceae, the existence of physiologic races has been established in most of the more important economic smuts. Information is accumulating concerning their number and geographic range, two problems which bear directly on the breeding of crop plants for particular localities. The subject would furnish material for another paper, but I intend only to refer to one aspect of the problem which arises out of the present discussion, namely, the status of physiologic races in the Ustilaginales. This term, or some equivalent, has been applied to chlamydospore collections which have given consistent results when tested for pathogenicity on differential host varieties. In diseases like smut of oats and bunt of wheat, the normal method of propagation is to dust the grain of an appropriate variety with chlamydospores, germinate it under suitable conditions and harvest the resulting crop, taking precautions at all stages to prevent contamination. Thus the whole life cycle of the fungus is completed annually, and, as we have seen, this involves segregation, followed by a nuclear fusion. Does the collection of spores which constitutes a physiologic race come through this process unchanged? In other words, are they pure-breeding populations? Dillon Weston (1932) showed that the pathogenicity of some collections of bunt could be completely changed in two or three generations by repeated propagation on a particular host variety; a race of bunt with marked virulence for that variety was thereby selected from the original collection. Roemer & Bartholly (1933) record a similar result which they describe as the modification of aggressiveness, and Nicolaisen's work (1934, 1935) with the oat smuts led him to conclude that a given smut collection consists of a population of morphologically distinct, mostly heterozygotic biotypes. Morphology refers in this connexion to the cultural characteristics of haploid lines.

Some screening experiments with oat-smut collections, described in 1927 as distinct physiologic races, have been carried out at Aberystwyth, and the pathogenicity of the same collections was examined after building up new populations from single chlamydospores. The data support the view that chlamydospores may be heterozygotic for pathogenicity, but screening did not always have the effect of separating out component types. It seems that the

heterozygous condition can persist through several generations of chlamydospores (Sampson & Western, 1938). Observations by Western have some bearing on this point. He concluded that under natural conditions the organ of penetration is usually the promycelium, and this automatically limits the chances of hybridization between the sporidia arising from distinct spores. The multiplication of gametes which takes place so readily in culture and offers a ready means of crossing may perhaps not play such an important part in the natural life cycle of a smut (Western, 1937). Walter (1934) in his study of the penetration of *Zea mais* by *Ustilago Zeae* states that the germ tube usually penetrates the host directly, sporidia playing no part in the process.

The conclusion has been reached that collections of chlamydospores, even if they have their origin in single spores, are not of necessity single genotypes. The same is true of many physiologic races of rusts, as Newton and Johnson (1932) have pointed out, but since rusts are usually maintained in cultivation by successive uredospore generations ("dikaryotic clones" according to Stakman *et al.* (1930)), their hybrid character is not often brought forcibly to notice by changes in pathogenicity or other characters during the normal course of experimentation.

Physiologic races of smuts are usually identical in morphology, though it is sometimes possible to find a difference in size of spore which statisticians might regard as significant! Yet even without this biometrical character physiologic races are given specific rank in many genera. As Butler (1926) said in his paper on the delimitation of species, "in smuts the distinction by hosts is almost complete". In recent years the multiplication of species on this criterion has gone on apace. The present situation has been discussed by Ciferri (1932) from his own extensive knowledge of the Ustilaginales. He takes as an example *Entyloma Calendulae* (Oud.) de Bary *sensu lato*, a species which attacks the Compositae, and which is now split into twenty-five species, no fewer than thirteen of which differ only in supposed (not always proven) host specialization. To revise the classification of the Ustilaginales retaining the Linnaean binomial only for types clearly distinguishable on morphological characters would involve a vast amount of labour, but it would be a task which would earn the gratitude of all future mycologists interested in the smut fungi.

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THE LIFE HISTORY OF *RAMULARIA VALLISUMBROSÆ* CAV. ON *NARCISSUS*

By P. H. GREGORY

Seale-Hayne Agricultural College, Newton Abbot

(With Plate I and 33 Text-figures)

I. INTRODUCTION

RAMULARIA VALLISUMBROSÆ CAV. causes the white mould disease of *Narcissus*. It is widely distributed on different species and varieties of *Narcissus* and assumes epidemic proportions in the south-west of England in districts devoted to the outdoor production of *Narcissus* flowers. I have investigated the fungus in this area, and an account of the disease and of its control is in preparation. This paper describes the life history of the fungus.

2. HISTORICAL REVIEW

The *Narcissus* white mould fungus was first described by Cavara (1899) as follows:

“*Ramularia Vallisumbrosæ* n.sp. (fig. 1-2. planche cxcvii).

“Amphigena; maculis oblongis, initio flavo-ochraceis, pruina albida conspersis; hyphis fasciculatis, e stromate mycelico erumpunt, ortis, subtilibus, cylindraceis, simplicibus vel ramosis, septulatis, albidis; conidiis inaequalibus, cylindricis, continuis vel 1-3 septatis, utrinque plus minus truncatis, intus granulosi, concoloribus. 14-44 $\times$ 4 μ .”

“*Habit.* In foliis Narcissi Pseudonarcissi, biflori, poetici, odorati, etc., Horti Botanici et Arboreti dendrologici Vallisumbrosæ (in Etruria).”

This description was copied by Briosi & Cavara (1900), and by Saccardo (1902). Lindau (1907) translated Cavara's description, but added the suggestion that the conidiophores emerge through the stomata.

A few years after the discovery of the fungus in Italy, a species of *Ramularia* on *Narcissus poeticus* was found in England by Chittenden (1906), and named *Ramularia Narcissi*; but in 1912 Chittenden concluded that his fungus was *R. Vallisumbrosæ* Cav.

In the meantime, Boudier (1901) described a fungus from *Narcissus*

foliage in France which he named *Cercospora Narcissi*. The following is his description:

“*Cercospora Narcissi* n.sp.

“Alba aut albida, 100–150 μ alta, effusa, fasciculata, amphigena, in partibus foliorum mortefactis ochraceis aut ochreofuscis parasitans, sporulis elongatis, inverse cylindrico-clavatis.

“Hyphae conidiferae fasciculatae, breves, 20 μ circiter longae, 2–3 crassae, hyalinae, continuae, intus granulosae, cylindricae sed ad apicem undulosae vix attenuatae. Sporulae majores, 50–130 μ longae, 4–5 μ latae, 3–8 septatae, hyalinae, intus granulosae praecipue ad basim, ad apicem saepius attenuatae, sed etiam breviores, cylindricae.

“Ad folia *Narcissi poetici* culti quae enecat; Montmorency, 1898 et 1899. Etiam Peronnas prope Bourg (Ain) unde misit Dom Clerc.”

In compiling *Ramularia Narcissi*, Saccardo (1913) suggested that *Cercospora Narcissi* is a more highly developed form of *Ramularia Vallisumbrosae*. Stevenson (1926) also suggested that *Cercospora Narcissi* Boud. and *Ramularia Narcissi* Chit. might be synonyms of the fungus which was cited incorrectly as “*Ramularia vallambrosae* Br. & Cav.” This synonymy appeared more probable when the Ministry of Agriculture and Fisheries (1934) reported that the fructifications of a fungus apparently identical with *Cercospora Narcissi* Boud. are occasionally found mixed with the *Ramularia* on leaves of *Narcissus* affected by white mould.

The sclerotial stage of *Ramularia Vallisumbrosae* was first reported by Beaumont (1926), who observed on withered leaves numerous very minute black bodies which probably represented the resting stage of the fungus, but which contained no spores. Work with pure cultures (1929) at the Ministry of Agriculture and Fisheries, Plant Pathological Laboratory, Harpenden, showed that these black bodies really belonged to this *Ramularia* and not to any secondary fungus accompanying it. On material collected during a severe outbreak of the disease in Oregon in 1930 McWhorter (1931) noted the presence of sclerotium-like structures which develop in great numbers on the dying leaves after conidial formation has ceased. He also stated that their behaviour in culture indicated that the structures were the primordia of pycnidia, which under suitable cultural conditions could produce multiseptate pycnospores. The production of filiform spores from sclerotia was recorded by Gregory (1936).

3. DESCRIPTION OF THE WHITE MOULD DISEASE

Primary lesions of white mould may be found when the young *Narcissus* leaves are only from three to four inches above the ground. They begin as small, sunken, grey streaks near the tip of the leaf on

the outer surface. In time they enlarge and appear as yellowish brown necrotic spots covered with a short, dense layer of buff to chalky white conidia, usually disposed in tufts on sporodochia. In certain varieties, the necrotic area is surrounded by a narrow dark green band. Outside the sporing area is a broad indefinite region of straw-yellow tissue, which fades off gradually into the normal green colour. In suitable weather secondary lesions soon appear lower down the leaf and on nearby leaves and flower stalks.

As the season advances, sclerotia develop on the older parts of the lesion. Usually there is a particularly dense zone of sclerotia around the periphery of the sporulating area of each lesion, but as the tissues wither a sparser population of sclerotia develops on the whole aerial part of the leaf. Sclerotia may be found on the leaf-tip lesion while the rest of the leaf is still green, but the chief development takes place when the leaf has withered. Sclerotia develop only on the portion of the leaf above ground and a fraction of an inch below soil level; neither lesions nor sclerotia occur on the bulb itself. The sclerotia are organs of aestivation.

White mould lesions have not been observed on the two sheathing leaves which enclose the foliage leaves while pushing through the ground, and it is therefore probable that reinfection of the plant in the spring is brought about by air-borne spores produced from the sclerotia, rather than by mycelium growing out from the sclerotia to attack the shoot below ground-level. Klebahn (1918), and Killian (1923, 1926, 1928) have shown that species of *Ramularia* vary in the behaviour of their resting bodies, but that the production of conidia or ascospores above ground is usual. The present study of *R. Vallisumbrosae* supports this generalization. In its natural habitat, where the *Narcissus* bulb is never dug up and replanted, the sclerotia formed in the withered leaves of the previous season are favourably placed to bring about reinfection of the new leaves. The sclerotia have usually been regarded merely as perennating organs, but it is probable that when leaves are scattered in the wind or distributed in some other manner the sclerotia may help to disseminate the fungus.

4. INFECTION

Microscopical examination of portions of leaf tissue taken from below actively sporulating white mould lesions shows that the conidia tend to lodge in the longitudinal grooves of the cuticle, where, on germination, they produce hyphae which enter through the stomata of the host. On one area of less than 12 sq. mm. of healthy leaf below a sporulating lesion no less than thirty-three germinating conidia were observed, sending infection hyphae through stomata. Direct penetration of the cuticle by germ tubes has not been observed, but this would be more difficult to see than stomatal penetration,

The infection hypha is narrow and straight but swells out somewhat on passing between the guard cells of the stoma. Once within the substomatal chamber the infection hypha begins to branch and to extend down between the palisade cells in various directions so that an approximately hemispherical thallus is formed.

Intermediate stages in the infection process have not been followed as they cannot be identified with certainty in field material until shortly before conidia are formed. It is, however, possible to follow the process of extension of an established lesion which can positively be attributed to *Ramularia Vallisumbrosae* by the conidia produced at its centre.

The broad indefinite yellow zone which usually surrounds the spore-producing area lies beyond the periphery of the mycelium. The palisade cells in this zone show various degrees of contraction of protoplast and collapse of cell wall. At the edge of the mycelium the palisade cells are completely disorganized and their walls are not easily visible. Similar changes occur in the epidermal cells but are less easily distinguished owing to the absence of chloroplasts, and to the greater rigidity of the cell walls.

Transverse sections of the leaf show that near the edge of the thallus the radial walls of the epidermal cells crumple up and their thickened outer walls collapse upon the somewhat thinner inner walls (Text-fig. 1). The lumen of the epidermal cells is thus almost obliterated. The mesophyll also collapses, and, as a result, the portion of the leaf invaded by the fungus is greatly reduced in thickness.

The vascular bundles do not appear to be penetrated by the mycelium, but hyphae can be observed which have crossed along parenchymatous strands from one side of the leaf to the other. The mycelium consists of more or less sinuous hyphae which are at first uniform in diameter, but in the older parts of the lesion, where the walls of the hyphae often become brownish, the cells are barrel-shaped between their closely placed septa.

In the host tissue the hyphae are mainly intercellular. Death of the host cells takes place well in advance of the pioneering hyphae, and the mycelium ramifies between and along the dead host cells. The only hyphae which do not remain intercellular are some which enter the cell cavities of the collapsed epidermis, and are sometimes embedded in the cellulose wall (Text-figs. 2, 3). It is from hyphae within the epidermal cells that the sclerotia develop later.

The mycelium is not an irregular tangle, for the hyphae tend to be associated into plates growing in planes parallel to the epidermis. The chief plane of advance is below the palisade layer. From these plates hyphae grow up between the disintegrating cells of the palisade layer. An important plate lies between the palisade and the epidermis,



Text-figs. 1-4. Invasion of epidermis and development of subcuticular stroma in transverse section of leaf lesions on *Narcissus* Silver Chimes. Stained cotton blue in lactophenol.

Text-fig. 1. Hyphae present between palisade and epidermis near margin of thallus, showing reduction in lumen of epidermal cells.

Text-fig. 2. Hyphae in lumen of epidermal cell.

Text-fig. 3. Hyphae beneath cuticle.

Text-fig. 4. Fundament of sporodochium pushing up cuticle.

Text-fig. 5. Longitudinal section near edge of thallus in leaf of *Narcissus* Golden Spur. Stained cotton blue in lactophenol: (a) branching of tip of hypha leading from subepidermal mycelium; (b) a lenticular sporodochial fundament beneath cuticle.

Text-fig. 6. Transverse section of young subcuticular sporodochium bearing scoleospores on leaf of *Narcissus* Golden Spur, collected January 1935.

Text-fig. 7. Conidiophores on leaf spot on *Narcissus poeticus ornatus maximus* collected dry (see text), Isles of Scilly, April 1938: (a) rounded growing tip of hypha; (b) scar on conidiophore from which conidium has become detached. Mounted in glycerine jelly after soaking in dilute potash.

Text-fig. 8. Same material as Text-fig. 7, showing (a) faceted, and (b) geniculate conidiophore, according to position of scar.

Text-fig. 9. Portion of aerial mycelium and conidiophores in hanging-drop culture on Czapek's agar sketched *in situ*: (a) at time of first observation; and (b) forty-eight hours later to show acropetal development of chains of amero-spores.

Text-fig. 10. Transverse section of a sporodochium developing beneath cuticle covering guard cells of stoma on lower surface of leaf of *Narcissus* Silver Chimes. Stained cotton blue in lactophenol.

(All figures are from *camera lucida* drawings, except Text-fig. 9.)

This system communicates, by hyphae running between the epidermal cells (Text-fig. 5*a*), with the sporogenous system which is entirely subcuticular.

5. DEVELOPMENT OF SPORODOCHIA

Observations on whole leaves and portions of stripped epidermis suggested at first that sporodochia mostly arose in the substomatal chambers, because the groups of conidiophores, often found in close connexion with stomata, appear to emerge from between the guard cells. This view was expressed by Lindau (1907), who added to Cavares's (1899) descriptions of the conidiophores the question "in dem Spaltöffnungen?" Careful examination of sections of numerous specimens has shown that this view is erroneous, as the sporodochia usually arise below the cuticle covering an ordinary epidermal cell or a guard cell (Pl. I, fig. 1). Cavares's figure does not make this relationship clear, and suggests that the sporodochium has its origin within an epidermal cell, and not immediately below the cuticle. Sporodochia formed within epidermal cells have been found rarely in material collected in Cornwall. Very rarely a knot of hyphae has been seen filling a substomatal chamber and apparently sending conidiophores through the stomatal opening.

Killian (1926) found that the conidiophores of *Ramularia* can arise in different ways; in *R. Lamprosanæ* and *R. variabilis* they arise in the substomatal cavity, while in most other species they break through the epidermis. In *R. variabilis* both modes of sporulation are found in succession. *R. Vallisumbrosae* belongs to the second of these three classes.

The development of a sporodochium can be traced most clearly in longitudinal sections such as that depicted in part in Text-fig. 5. A forward-running hypha in the mycelial plate lying immediately below the epidermis produces a branch which pushes its way up between two epidermal cells. Just beneath the cuticle the ascending hypha branches. So far as can be gathered from the examination of a number of sections, it is probable that each sporodochium arises by the subcuticular branching of a single hypha of this sort, by means of which it remains in communication with the subepidermal mycelium. Subsequent stages in the growth of sporodochia are shown in Text-figs. 4, 6 and 10.

The hyphae which initiate the sporodochia push up between the epidermal cells and spread laterally between the cuticle and the outer cellulose wall of the epidermis (Text-fig. 5*a*). They then produce swollen vertical branches which force the cuticle away from the rest of the epidermis. This separation appears to be entirely mechanical as no dissolution of cuticle or wall is observed at this stage. The

cuticle is pushed up and the rest of the epidermis pushed down so that just before it ruptures the cuticle the sporodochium has the form of a biconvex lens (Text-fig. 5*b*).

In a precisely similar manner the sporodochia associated with stomata arise between the cuticle and cellulose part of the wall of one, or sometimes both, of the guard cells (Text-fig. 10). The extrusion of the conidiophores from beneath the cuticle along the edge of the guard cell then gives the false impression, when examined from above, that they arise from the substomatal chamber.

There appears to be no subcuticular mycelial net connecting individual sporodochia. Each sporodochium is isolated from neighbouring sporodochia laterally, but each is independently connected with the subepidermal mycelial plate lying below.

Eventually the cuticle above the sporodochium is ruptured by the elongation of the vertically placed hyphae (Pl. I, fig. 1), and further growth of the hyphae at the base of the sporodochium produces a plectenchymatous mass (Text-fig. 6). Sporodochia may grow at their circumference and become confluent with adjacent sporodochia, so that sporogenous patches may eventually cover a wide area on the leaf.

6. CONIDIA AND CONIDIOPHORES

The conidia and conidiophores vary greatly in different specimens of white mould and even on different parts of the same leaf, so that no precise description of their dimensions can be given. Numerous observations on hand sections through lesions in all stages have shown that the length of the conidiophore varies inversely with the length and number of the conidia which it bears. *Ramularia Vallisumbrosae* can produce hyaline conidia showing continuous variation in form from amerospores, through phragmospores to scolecospores, corresponding with the form genera *Ovularia*, *Ramularia* and *Cercospora*. The conidiophores associated with each type require separate description, but no real boundary can be recognized between one type and another. In these descriptions the names of spore types used by Mason (1933, 1937) have been adopted.

Scolecospores. Early in the season, particularly on primary lesions, the sporodochia produce scolecospores. The closely packed, vertically arranged hyphae of the young sporodochium, which have pushed up through the cuticle, continue to grow out into the air as unbranched hyphae whose distal parts are transformed into conidia. At this stage a conidium can be distinguished from the end of a conidiophore which has shed its spore, by its apex, which is rounded and has a wall of uniform thickness, while the apex of the conidiophore is abruptly flattened and somewhat more refractive than the rest of

the hypha (Text-fig. 7). When the sporogenous hypha has reached a length of $40-100\mu$, the distal part is cut off from the basal portion of the hypha just above the surface of the leaf. This terminal portion of the hypha is finally detached as a scolecospore. The scolecospores measure $40-95\mu$ in length, their rounded bases are $2.5-3.5\mu$ across, they often taper to 2μ across at the apex, and have from three to seven septa when mature.

As most of the conidia are cut off from their conidiophores at about the same level, the ends of the conidiophores appear as a level hymenium forming a cushion slightly raised above the epidermis when all the conidia have been shed. These conidiophores occupy the upper half of the sporodochium as straight, unbranched, non-septate hyphae. When each has cut off one scolecospore some of the conidiophores are left empty and presumably cease to function; but others may produce a second spore which is formed beside the first in a manner seen more clearly and regularly in the conidiophores bearing phragmospores described below. Scolecospores have never been observed in chains.

Phragmospores. In the production of phragmospores the tip of the hypha projecting from the sporodochium cuts off a relatively short conidium, so that when the spores have been shed the truncated ends of the conidiophores project $10-50\mu$ beyond the leaf, and often radiate from the sporodochium at various angles. The non-septate conidiophores which bear phragmospores are straight, unbranched, and measure $10-50 \times 3-4\mu$.

Frequently several phragmospores are produced on the same conidiophore. No second conidium has been observed growing from the truncated end of the conidiophore or "scar" whence the first conidium became detached. The scolecospores and phragmospores are thus *terminus thallospores*. When a second phragmospore is produced, a new growing tip arises beside the scar, a hypha is formed and its end is cut off by a septum before being liberated as a conidium. The septum may arise in either of two positions: (1) at the same level as the former scar, giving a faceted appearance to the conidiophore (Text-fig. 8a); or (2) at a short distance above the scar, when the conidiophore becomes geniculate (Text-fig. 8b). Both these conditions may be seen in the production of scolecospores as well as in phragmospores. Phragmospores are occasionally catenulate, as sometimes a second conidium is formed from the apex of the first. There is no reason to suppose that a conidiophore which has produced scolecospores at one stage cannot later give crops of phragmospores if food is available under appropriate conditions.

Anerospores. Short non-septate conidia, measuring $3-10 \times 3-4\mu$, are also formed. They are not immature individuals of the larger spore types, as the ultimate length of the conidium is practically

determined when the septum is laid down delimiting the conidium from the conidiophore. Amerospores are most numerous under very moist conditions. In a humid atmosphere each hypha emerging from the sporodochium tends to become long, and to cut off a short, broad, non-septate conidium at its tip. Often before this is shed another growing tip is established beside the base of the first conidium and another long hypha is produced which again cuts off an amerspore at its apex. The process may be continued and the scars on the conidiophore are relatively far apart. Hyphal fusions are frequently observed between these long conidiophores. This type of sporulation may be further complicated under humid conditions by catenulation. At the end of one amerspore another may develop in acropetal succession, leading to the formation of chains of conidia of the *Ovularia* type. These agree with the definition of *dry blastospores* given by Mason (1937, p. 82). This extreme development is seen in hanging drop cultures (Text-fig. 9a, b) in which long chains of amerspores are produced.

The process of conidium formation may be summarized in general terms which bring out the relationship between the different types. A hypha lying in the surface hymenial layer of the sporodochium continues to grow into the air as a simple unbranched non-septate hypha. Cessation of growth at the hyphal tip apparently coincides with the laying down of a septum which cuts off the conidium from the conidiophore. According to the distance of this first septum from the tip of the hypha, a scolecospore, phragmospore, or amerspore develops. What determines the position of this abscission septum we do not know, but it is noteworthy that the scolecospores are narrower than the phragmospores and amerspores.

In Mason's terms all the conidial forms are "dry spores". When a leaf bearing conidial fructifications is shaken over a sheet of black paper a cloud of conidia becomes detached. The conidia are probably distributed in nature principally by wind.

Evidence of the relationship of these conidia of different lengths is based on (1) the examination of a large number of collections of white mould of *Narcissus*; (2) single or multispore isolations from representative collections; and (3) inoculations carried out with these cultures.

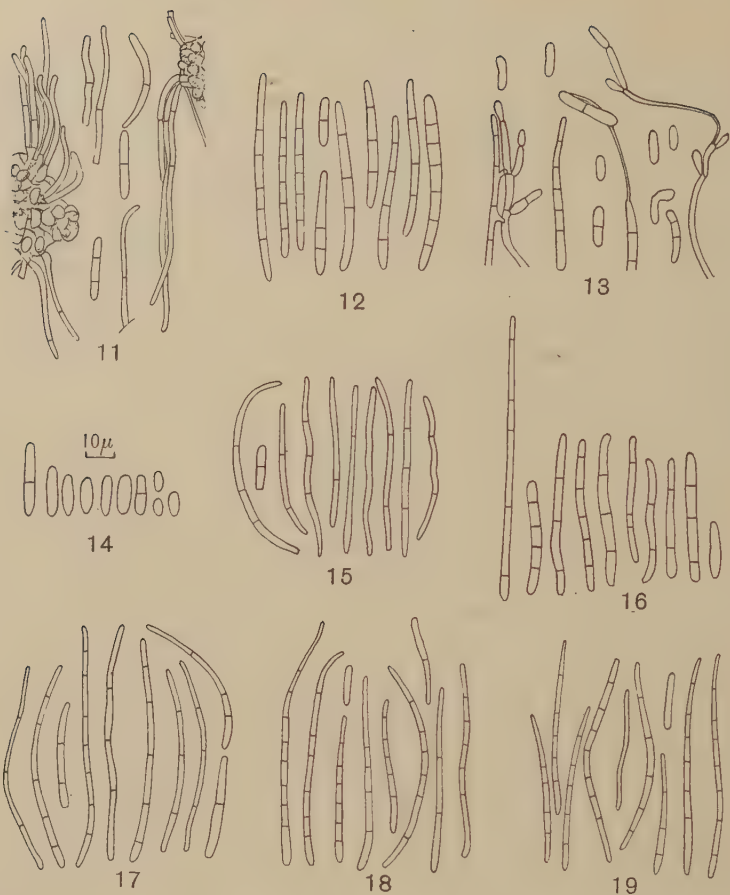
7. OCCURRENCE OF THE DIFFERENT FORMS OF CONIDIA ON LEAF-SPOTS

In January 1935 cultures now known to be typical of *Ramularia Vallisumbrosae* were obtained from multispore isolations from primary white mould lesions collected in the Isles of Scilly. These lesions bore hyaline scolecospores on sporodochia, but phragmospores of the

Ramularia type were absent. Subsequent collections of the fungus usually bore conidia of *Ramularia* only. In the winter of 1936-7 it was realized that when the specimens were being taken to the laboratory in closed tins they often developed a copious white layer of conidia on the mature lesions, and that when examined they were not in the same condition as they were when collected. A series of collections was therefore brought to the laboratory between sheets of absorbent paper in as dry a state as possible. It is believed that this material was not in a favourable condition for the extensive growth of the fungus during transport, and that the conidia found when the partially desiccated leaves were examined were those which had been present on the foliage out of doors. Nearly all of the thirty-four samples examined critically showed a mixture of spore forms; twenty-eight were producing scolecospores, twenty-two phragmospores and eight were producing amerospores.

The examinations show conclusively that in the field scolecospores are at least as common as phragmospores, and that only exceptionally moist conditions favour the development of amerospores. If mycologists had not formed the habit of collecting in tins this fungus would probably never have been referred to *Ramularia*. The fact that scolecospores are almost the sole spore form found in the early months of the year may possibly show the effect of lower temperatures or differences in nutrition in determining the form of spore produced, but circumstantial evidence points to humidity as one of the controlling factors. Authentic material of *Ramularia Vallisumbrosae* issued by Briosi & Cavara (1900) and preserved in the herbarium of the Imperial Mycological Institute, Kew, was somewhat deficient in conidia. Those present were mainly phragmospores, but a few not yet detached from their conidiophores were probably scolecospores (Text-fig. 11). The specimens examined left no doubt that the fungus present on *Narcissus* in England is *Ramularia Vallisumbrosae* Cav.

Observations illustrating the effect of high humidity in favouring the development of broader and shorter spores include the following. On two dates, separated by ten days, infected leaves were collected from the same beds of *Narcissus*. The earlier collection which was kept in a closed tin had some phragmospores and many amerospores. The later collection, which had been brought back dry, bore only scolecospores. This difference probably indicates the effect of confining sporodochia in a humid atmosphere. The converse observation was made on foliage of the variety *Victoria*, which when first examined after transport in a dry condition had scolecospores (Text-fig. 12), from which three monoconidial isolates were prepared and used in inoculations nos. 20-23 in Table II. After a few leaves had been kept on moist filter paper in Petri dishes for forty-eight hours a white layer of phragmospores and amerospores developed



Text-figs. 11-19. Conidia from various collections of *Ramularia Vallisumbrosae*, drawn from mounts in glycerine jelly (except Text-fig. 11 in balsam, and Text-fig. 12 in glycerine). Mag. 500 $\times$, reduced from 1000 $\times$ in original drawings.

Text-fig. 11. Phragmospores, and possibly, scolecospores from authentic material of *R. Vallisumbrosae* (Briosi & Cavara (1900)).

Text-fig. 12. Conidia from leaf spots on *Narcissus* Victoria, collected dry (see text), Truro, 19 March 1937.

Text-fig. 13. Same collection as Text-fig. 12, but drawn after keeping in closed, moist tin box for forty-eight hours.

Text-fig. 14. Conidia from leaf spots on *Narcissus* Golden Spur growing in unusually moist situation, St Mary's, Isles of Scilly, 1 April 1937.

Text-fig. 15. Conidia from leaf spots on *Narcissus* Golden Spur, collected dry (see text) 19 March 1937.

Text-fig. 16. Conidia from leaf spots on same stock of plants as Text-fig. 15, but collected dry, 7 May 1937.

Text-fig. 17. Conidia from leaf spots on *Narcissus* var. *Horsfieldii*, collected dry, 23 March 1937.

Text-fig. 18. Conidia from sclerotia on leaves of *Narcissus* collected Penberth, Cornwall, May 1935, sprouted at Newton Abbot, Devon, 17 February 1936.

Text-fig. 19. As Text-fig. 18, but sprouted 27 February 1936.

(Text-fig. 13). A culture was started with a single phragmospore from this material and used in performing inoculation 24 in Table II. Finally, a deliberate search was carried out in very moist places for leaves bearing amerospores alone. In a garden with a south-west aspect at Porth Cressa, St Mary's, Isles of Scilly, material bearing amerospores with occasional phragmospores only was discovered. The amerospores are illustrated in Text-fig. 14. A culture was made with a single non-septate conidium from this material and used in performing inoculation no. 25 in Table II.

The scolecospore form, shown in Text-figs. 15-17, is probably the normal and most highly developed conidium found on infected leaves in the field. The factors which determine the development of these spore forms have not been elucidated, but they would repay analysis and provide a contribution to our knowledge of the *Fungi imperfecti*. It is certain that the smaller forms are not immature conidia which later develop into scolecospores. On the contrary the scolecospore is the forerunner of the amerospore in the history of any lesion.

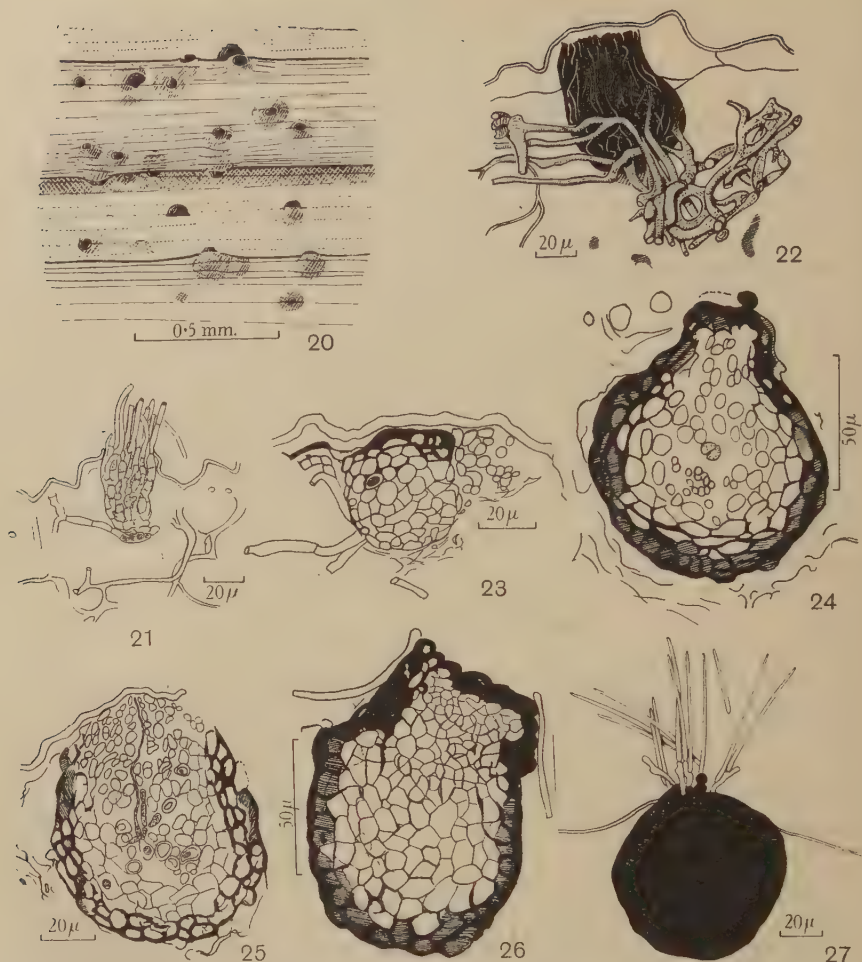
8. SCLEROTIA

As seen with a hand lens, the sclerotia begin as brown bodies which rapidly darken to black. They consist of two parts which may be referred to as a roughly spherical "body" embedded in the leaf, and a short "neck" which projects through the cuticle (Text-fig. 20). Their diameter ranges from 70 to 140 μ . The number formed varies considerably, but at their densest, near the periphery of a lesion producing spores, there may be upwards of ten sclerotia per square millimetre on each side of the leaf.

The sclerotia are formed on the same part of the leaf as are the sporodochia. As the leaf withers the production of sporodochia ceases and that of sclerotia begins. When the leaves have finally died down the sclerotia are densely concentrated on and around the peripheries of the old conidial areas, and sparsely but evenly distributed over almost the whole of the remainder of the aerial portion of the leaf.

Occasionally structures are found which appear to be intermediate between true sporodochia and sclerotia (Text-fig. 21). They are compact hyaline stromata, usually spherical to cylindrical, embedded in the leaf but sending tufts of conidiophores through the cuticle. They differ from typical sporodochia in their extensive stromata, and from sclerotia in their hyaline outer layers and production of conidia without a resting period. They are probably derived from sclerotial initials which have for some reason failed to develop into sclerotia.

Development of sclerotia. The early stages in the development of the



Text-fig. 20. Surface view of withered leaf in summer showing immersed sclerotia with necks protruding through cuticle.

Text-fig. 21. Body intermediate between sporodochium and sclerotium, with conidiophores formed June 1936 on leaf spots on *Narcissus poeticus recurvus*.

Text-fig. 22. Transverse section of leaf of *Narcissus poeticus recurvus*, June 1936, showing system of dark thickened hyphae connecting sclerotium with rest of mycelium in leaf.

Text-fig. 23. Stage in development of sclerotium showing plectenchymatous stroma whose cells nearest the cuticle are becoming sclerotic. In transverse section of leaf of *Narcissus* var. Golden Spur, Penberth, Cornwall, May 1935.

Text-fig. 24. Incompletely developed sclerotium, collected Truro, October 1935, perhaps the fundament of a perithecium.

Text-fig. 25. Another incompletely developed sclerotium with organ resembling a trichogyne in venter and neck; on leaf of *Narcissus* var. Golden Spur, Penberth, Cornwall, May 1935.

Text-fig. 26. Longitudinal section of sclerotium in dormant condition of aestivation, Truro, October 1935.

Text-fig. 27. Scolecospores produced on geniculate conidiophores in neck of sprouting sclerotium, mounted in glycerine after clearing in dilute potash. The neck in this specimen was somewhat downturned and therefore not shown in drawing.

sclerotia have not been closely followed, owing to insufficiency of material at the right stages.

It is not clear whether a sclerotium arises by the branching of one or several hyphae, but a mature sclerotium has a root-like system of wide dark hyphae connected with it through which presumably it is supplied with food reserves (Text-fig. 22). The sclerotium usually begins as a small hyphal knot in the lumen of an epidermal cell (Pl. I, fig. 2) or less frequently at a point on the mycelial net near the disintegrating palisade layer. At this stage most of the tissues in the mesophyll of the leaf are unrecognizable, and the spaces left by the disintegrated cells are filled with a loose web of hyphae. By the time the sclerotia are well differentiated and have acquired their black carbonaceous rind, the cell walls of the epidermis are gone, leaving only the cuticle. This persists, holding the sclerotia in position for as long as the remains of the leaf can be recognized. In most stomata the whole internal tissue seems to become united to form a pseudoparenchyma. This change takes place during May and June and the structure may then be regarded as a well-formed sclerotium. The internal tissues normally show a differentiation of a remarkable kind which does not appear to have been described in other species of *Ramularia*. The body is filled with large polyhedral cells whose thin walls become somewhat carbonaceous. In the mature sclerotium these cells are filled with large globules. When sclerotia are crushed the globules are exuded and quickly run together. The globules stain red with Scharlach R, and reddish with Nile blue sulphate, thus indicating the presence of a neutral fat. In sections the cells of the body do not stain with the usual cytoplasmic stains such as haematoxylin and eosin, cotton blue, and methylene blue, and the body may perhaps be regarded tentatively as a dead storage organ, though it is possible that the cells retain a thin lining of cytoplasm.

The neck of the sclerotium, however, with its single-layered wall of carbonaceous cells consists of a cylinder of small living cells. The cells are full of protoplasm and are arranged in vertical columns which extend up to the single layer of small blackened cells covering the top of the sclerotium. This tissue may be likened to a meristematic layer in the higher plants, and it is from this cylinder that the conidiophores are produced in the spring (Text-fig. 26 and Pl. I, fig. 3).

The sclerotia reach this state of differentiation by June and retain it as the resting form by means of which the fungus passes the summer. During the summer if leaf lesions bearing sclerotia are kept moist no fresh conidia are produced, and the sclerotia normally show no further change. The fungus evidently aestivates in a condition of complete dormancy. It may be surmised that the function of the carbonaceous coat is both to protect the living tissue within from

damage, and to prevent it from being started prematurely into activity before its host has broken the ground. The nature of the stimulus which eventually starts the sclerotium into activity at the appropriate season is unknown.

In some sclerotia the hyaline hyphae within the body may remain thin walled and apparently discrete throughout the summer and winter (Text-fig. 24). The fate of these sclerotia is not yet known; perhaps they represent the initials of a perithecial stage at present unknown. This possibility is supported by the occurrence in some of these sclerotia of hyphae strongly resembling trichogynes. In certain of these incompletely developed bodies thin hyphae have been found growing up from large cells near the base towards the neck. One of these, illustrated in Text-fig. 25, shows resemblance to the trichogynes figured by Higgins (1936) in *Mycosphaerella tulipiferae* (Schw.) Higgins, and by Jenkins (1938) in *M. arachidicola* Jenkins. Definite proof of the connexion of sclerotia of *Ramularia Vallisumbrosae* with the supposed trichogyne is lacking and the evidence is purely circumstantial. So far no ascus stage has been found in the life cycle of *R. Vallisumbrosae*. On the other hand, these immature bodies may be sclerotia which have failed to obtain adequate fat reserves.

Sprouting of sclerotia. To follow the story further, special precautions had to be taken, as by the autumn visible remains of the previous season's leaves have usually disappeared from the surface of the *Narcissus* beds through weathering, cultivation and worms.

Simple methods were found most satisfactory for exposing small quantities of leaves to weathering during summer, autumn and winter. In one series of observations medium-sized flower pots were filled with fine soil, leaves bearing sclerotia were placed directly on the surface of the soil, and a layer of coarse muslin was tied over the mouth of the pot. In other experiments the hole in the pot was merely plugged with glass-wool, the pot filled with leaves, and covered with muslin as before. The pots were exposed in the open at a number of centres in convenient places where they were not likely to be disturbed, such as on a stone hedge, or partly sunk in the soil in a bed of daffodils. For more complete protection some leaves were also placed in test-tubes which were laid on the soil in flower pots as before. A still simpler method consisted in making a pile of *Narcissus* leaves bearing sclerotia in an out-of-the-way corner, and in spite of wastage from various causes enough leaves remained to provide ample material for study.

Infected leaves from several localities were exposed under various conditions at five centres during the years 1936-8. During the first season specimens were collected for examination from each centre at approximately monthly intervals during summer, autumn and

winter, but subsequently a few collections during the middle of the winter were relied upon to determine the dates at which the sclerotia regained activity. The specimens from the various samples were examined with the microscope after teasing in water, after cutting microtome sections, or after keeping for a few days in the laboratory in a moist Petri dish.

The sprouting of sclerotia with the production of hyaline scolecospores was observed for the first time at the Isles of Scilly Experiment Station, on material collected on 22 January 1936. Subsequent examination of specimens collected at slightly earlier dates at Gulval, Penzance, and at Truro, showed that sclerotia were producing conidia at these centres also. Only one sclerotium sprouted during January in material kept at Newton Abbot. The most abundant sprouting of sclerotia took place at all centres during the middle of February, and by early March it was over. During the winter of 1936-7 sprouting was again observed at the various centres during the month of January, but in the following winter the earliest record was made on 20 December 1937, from the Isles of Scilly, and by 19 January 1938 abundant scolecospores were being produced in material exposed at Newton Abbot. The sprouting of sclerotia in January and February is appropriately timed for infection of the leaves immediately after they have come through the soil.

For the microscopic study of the fructifications, portions of withered leaves were teased in water or in cotton-blue lactophenol. Tufts of hyaline scolecospores could be seen around the necks of the sclerotia with the 4 mm. objective, the whole strongly resembling the pycnidium of a *Septoria* liberating filiform pycnidiospores (Pl. I, fig. 6). Closer examination showed that the conidia were not coming from a typical pycnidium but from conidiophores lining a shallow depression in the neck of the sclerotium. This method of development of the sclerotium is peculiar and does not appear to be known in other species of *Ramularia*. The conidia are produced on geniculate conidiophores and are of the *Cercospora* type (Text-fig. 27). Subsequent experience has shown that sprouting of the sclerotia may occasionally give rise to phragmospores or even to amerospores. Out of doors scolecospores are usually the only kind of spore found, but in moist dishes in the laboratory the shorter forms of conidia may predominate.

The scolecospores are hyaline, $25-105 \times 2.0-2.75 \mu$ (mean $56 \times 2.2 \mu$), straight or curved, rounded at the proximal end, tapering slightly at the apex, and with from three to seven septa which are often indistinct.

In order to get a precise knowledge of the various stages in the sprouting of sclerotia, a well-matured and weathered leaf was prepared for microtome sectioning in the following manner.

The leaf selected was taken from a sample collected on 7 January 1936, near Penzance. Examination with the binocular microscope showed that the sclerotia were as yet unsprouted, but were presumably ripe for development, as in another leaf of the sample almost all the sclerotia had already sprouted and showed empty depressions in the neck whence the spores had been shed.

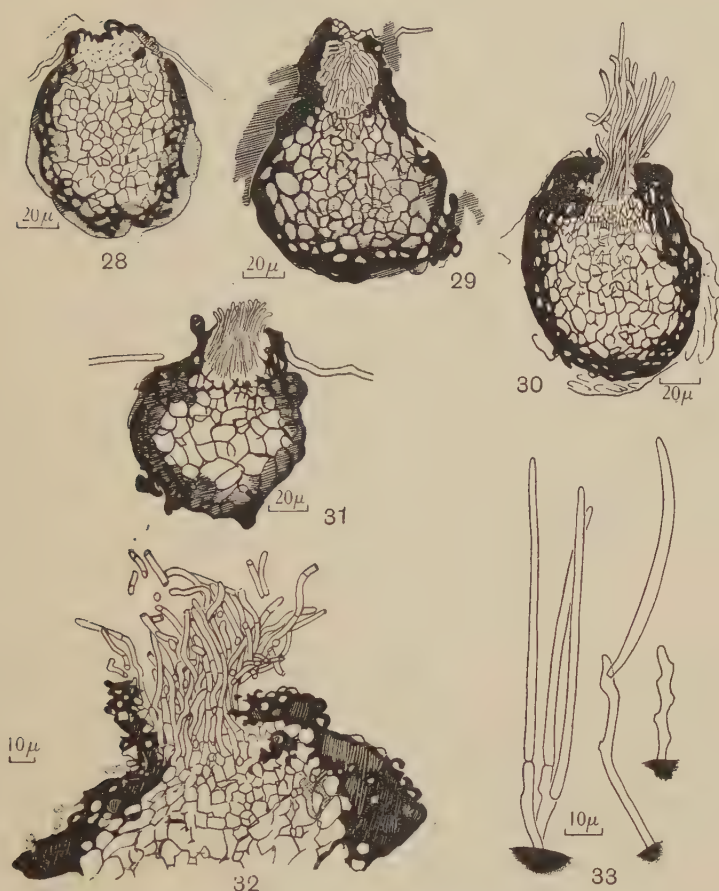
The leaf was cut into pieces about a centimetre in length and laid on moist filter paper in a Petri dish at room temperature. Then at intervals of twelve hours pieces of leaf were removed to Bouin's fixative. Portions of leaf bearing sclerotia were fixed after incubating for 0, 12, 24, 36, 48, 60, 72, 84 and 96 hours respectively. The later specimens bore copious tufts of white conidia visible to the naked eye before fixation (Pl. I, fig. 4). Microtome sections were cut of the sprouting sclerotia in different stages.

Several stages in development are illustrated in Text-figs. 28-32. Text-fig. 28 shows the condition of a ripened sclerotium before incubation; it is in fact indistinguishable from the resting stage which had endured since June of the previous year. After twenty-four hours (Text-fig. 29) the plug of "meristematic" tissue in the neck of the sclerotium had undergone a striking change. It was no longer a pseudoparenchymatous mass, but was organized into hyphae converging from the sides of the neck and top of the body towards the top of the sclerotium. The thin carbonaceous layers of cells covering the sclerotium had already been ruptured by the pressure of these outgrowing hyphae. After thirty-six hours (Text-fig. 30) these hyphae had grown out through a definite ostiole. A specimen fixed at forty-eight hours had not developed as far, while that fixed eighty-four hours after incubation showed signs of delimiting conidia (Text-fig. 32).

Microtome sections show how the layer of conidiophores lines a depression in the neck of the mass of sclerosed tissue, but a better idea of the disposition of conidia and conidiophores is obtained from teased preparations such as in Text-fig. 27, which shows a sclerotium with one or two geniculate conidiophores bearing conidia projecting from the shallow depression. Text-fig. 33 shows at a somewhat higher magnification conidiophores bearing conidia. Other conidia are shown in Text-figs. 18 and 19 alongside those found on the living leaf in spring for comparison. There is no doubt that the conidia formed on both living and over-wintered leaves are of similar type.

In the moist still air of a Petri dish the scolecospores can germinate *in situ* with the production of conidia of the *Ramularia* type.

When the conidia have been shed a cavity in the top of the sclerotium can be seen with a binocular microscope. Sections of such sclerotia show that all soft tissue has gone from the neck cavity,



Text-figs. 28-32. Stages in sprouting of aestivated sclerotia. Longitudinal microtome sections cut at intervals during incubation.

Text-fig. 28. Sclerotium in resting condition before incubation.

Text-fig. 29. Sclerotium after incubating twenty-four hours, showing arrangement of hyphae in cavity of neck.

Text-fig. 30. Sclerotium after incubating thirty-six hours, conidiophores protruding through neck of sclerotium.

Text-fig. 31. Sclerotium after incubating forty-eight hours, a less advanced stage than in Text-fig. 30.

Text-fig. 32. Sclerotium after incubating eighty-four hours, showing mass of conidiophores and conidia cut in various planes.

Text-fig. 33. Geniculate conidiophores and conidia from sprouting sclerotia, mounted in glycerine after clearing in dilute potash.

while the body to all appearances remains unchanged in morphology although the food reserves have disappeared.

Attempts were made to induce sclerotia which had discharged their conidia to produce a second crop of spores. Portions of leaves bearing sclerotia from which the first crop of conidia had been brushed, were kept in moist dishes for several weeks, but no further development took place. Evidently when conditions become favourable each sclerotium produces a single crop of conidia in the course of a few days, and the food reserves in the body are then apparently exhausted. If an unsprouted sclerotium is crushed in water between slide and cover-slip a copious exudation of fat droplets takes place, as noted on p. 37, pouring out through any break in the carbonized wall and apparently coming from the cells of the body. It was repeatedly noted, however, that when dry sclerotia which had discharged their crops of spores were crushed in a similar manner a mass of air-bubbles emerged from the body, but no fat globules could be found. The tissue of the sprouted sclerotium too had become exceedingly brittle. It was concluded that during summer the sclerotia are filled with a fat reserve which is exhausted in the production of a single crop of conidia. No further development has been noted and none would be expected.

9. ISOLATIONS

In earlier parts of this paper several conidial forms of *Ramularia Vallisumbrosae* have been described. These spore forms are: (1) scolecospores, phragmospores and amerospores produced on typical white mould lesions on the living *Narcissus* leaf; and (2) scolecospores, with occasional phragmospores and amerospores produced by the sclerotia after aestivation. This circumstantial evidence of association of these stages alone makes it highly probable that they are phases in the life cycle of one organism. The evidence from isolations and inoculations with single spore cultures is now given.

Mass isolations of conidia from white mould lesions collected in the field or after incubating in a moist dish for a day or two, readily gave cultures of a slow-growing fungus. Similar cultures were obtained also from mass transfers from sprouting sclerotia. Single conidia of the various forms, whether from sporodochia or sclerotia, also gave cultures of the same fungus. In all fifty-two isolates were obtained which were regarded as typical of *Ramularia Vallisumbrosae*. The various spore forms with which these cultures were started are shown in Table I.

Isolates have been grown on several nutrient media including Brown's glucose-asparagin agar, glucose agar, Czapek's agar, glucose-peptone agar, oatmeal extract agar, prune extract agar,

water agar, potato-plug, and also on Czapek's agar with crude lentil extract kindly supplied by Dr Lilian E. Hawker (1936). The following observations of the fungus on these media refer to room temperatures of 12–18° C.; no attempt was made to study growth at controlled temperatures. The number of isolations studied on each medium is given in brackets.

Variations occurred between certain isolates chiefly in the amount and colour of aerial mycelium which ranged from white to Indian red, and which sometimes showed sectoring in single-spore cultures. On any one medium differences between strains were also noted in the colour of the diffusible pigment which tinted the uncolonized portion of the medium. As shown in the following descriptions, this was redder in some strains than in others.

Glucose agar (glucose 20 g., di-potassium hydrogen phosphate 1 g., magnesium sulphate, 0.5 g., potassium chloride, 0.5 g., sodium nitrate, 2 g., agar 20 g., water 1000 c.c.) (two isolations).

Colony of staling type, flat, radial growth 2 mm. per month at room temperature, 4 mm. per month at 7–10° C.; aerial mycelium very sparse, white; immersed mycelium greenish brown, medium not noticeably pigmented; sclerotia loose, discrete, superficial, olive-black to black; conidia few, unseptate.

Glucose peptone agar (sucrose 30 g., di-potassium hydrogen phosphate 1 g., magnesium sulphate 0.5 g., potassium chloride 0.5 g., peptone (Witte) 10 g., ferrous sulphate 0.1 g., agar 20 g., water 1000 c.c.) (twenty-two isolations).

Colony of staling type, maximum radius on slants about 20 mm.; raised, convoluted to cerebriform; aerial mycelium short, white to Indian red; uncolonized medium becoming opalescent with diffusible venetian red pigment; reverse black; sclerotia black, discrete or confluent, 70–180 μ , heaped up to 4 mm. above medium; conidia few, unseptate, pink in mass.

Czapek's agar (sucrose 30 g., di-potassium hydrogen phosphate, 1 g., magnesium sulphate 0.5 g., potassium chloride 0.5 g., sodium nitrate 2 g., ferrous sulphate 0.1 g., agar 20 g., water 1000 c.c.) (six isolations).

Colony of staling type raised to about 1 mm. at centre; aerial mycelium sparse to abundant, short, white to madder carmine; sectoring observed; medium becoming opalescent with a diffusible pigment pale Venetian red to raw umber; sclerotia black, abundant, discrete; conidia unseptate, few.

Czapek's agar with 2 % crude lentil extract (six isolations).

Colony staling less readily than on Czapek's agar and contouring less pronounced; but all somewhat elevated at centre; aerial mycelium abundant, zoned, white at margin often with pale olive-yellow, raw umber to rose madder zones within; medium becoming opalescent with pale diffusible pigment burnt sienna or Venetian red; sclerotia abundant, black, discrete. Conidia indistinct.

Brown's glucose asparagin agar (sixteen isolations).

Colonies of staling type, maximum radius 5 mm.; slightly contoured; aerial mycelium very sparse, short tufted in concentric zones, white to Indian red; immersed mycelium black with no diffusible pigment in medium; sclerotia superficial, black, slightly raised in the mass, 45–130 μ ; conidia very few, unseptate.

Oatmeal agar (60 g. ground oats, warmed 70° C. for 15 min., agar 20 g., water 1000 c.c.) (sixteen isolations).

Colonies of non-staling type, growing at about 5 mm. per month, not raised above medium, superficial; aerial mycelium sparse, powdery to cottony, white to Naples yellow, present in short tufts over colony; immersed mycelium black; no diffusible pigment but reverse of slant a deep raw umber; sclerotia abundant, immersed, up to 360 μ diam., irregularly rounded; conidia unseptate, abundant, in branching chains.

Prune agar (40 g. prunes, 25 g. agar, 1000 c.c. water) (ten isolations).

Colony of non-staling type, slightly elevated at centre; aerial mycelium sparse in white tufts; immersed mycelium brownish black with numerous hyphal fusions; medium tinged deep Indian red by diffusible pigment; sclerotia numerous, black, up to 200 μ diam.; conidia an even pale burnt sienna to brown-ochre layer over centre of colony, unseptate.

Potato plug (four isolations).

Colony of staling type, sharply raised in form of a flat disk, 1 mm. thick, consisting of a stroma-like layer of confluent black sclerotia, covered by a short felt of white to pale brown-ochre aerial mycelium; medium not coloured by diffusible pigment; conidia unseptate.

The similarity of cultures derived from different sources, as summarized in Table I, is additional evidence of the relationship of the various spore forms and inoculations on to healthy leaves of *Narcissus* to be described below showed that the organism under consideration in culture was in fact the white mould fungus *Ramularia Vallisumbrosae*.

Table I. *Summary of isolations of Ramularia Vallisumbrosae*

Source and type of conidia	No. of single-spore cultures	No. of multi-spore cultures (mass isolations)*
From sporodochia:		
Scolecospores	3	4
Phragmospores	2	15
Amerospores	1	0
From sclerotia:		
Scolecospores	22	4
Phragmospores	1	0
Amerospores	0	0
Total	29	23

* The spore type from which multi-spore cultures were started cannot be precisely stated, but the predominant spore form in the material used is indicated.

10. INOCULATIONS

Inoculations of healthy *Narcissus* leaves with cultures and conidia of diverse origin were made at Seale-Hayne Agricultural College, Newton Abbot. Here the *Narcissus* white mould does not assume an epidemic form, though in some years a few patches of affected leaves can be found on three- or four-year-old beds of varieties such as *Narcissus poeticus recurvus*. This centre is therefore particularly suitable for carrying out experimental inoculations with *Ramularia Vallisumbrosae*, as there is practically no danger of accidental infection. In fact, over the whole period under review, although most of the inoculated leaves became infected, only one of the control leaves in one experiment showed infection by *R. Vallisumbrosae*, and this was evidently a secondary infection from a successful inoculation on an adjacent leaf.

All inoculations in 1936 and 1937 were made on the variety Golden Spur, but in 1938 a somewhat resistant variety, Grandis, was used instead. The plants growing in pots were inoculated by placing spore suspensions or portions of agar cultures near the tips of uninjured leaves. After inoculation it was found necessary to keep the leaves in a moist atmosphere, either by covering the pots with bell-jars, or by slipping each leaf into a wide test-tube which was afterwards plugged with cotton. The pots were left in a small insectary, with glass roof and wire gauze sides, in the shade of another building. Control leaves were either left untreated, or had small pieces of sterile agar placed on the leaf in a position similar to that of the piece of agar culture on the inoculated leaves. After three to four weeks, when white mould lesions developed, the leaf tips were removed and examined for the presence of conidia. In some instances cultures were made from the conidia, and the leaf tips were preserved for reference.

The first series of inoculations was carried out by placing scoleospores taken direct from sprouting sclerotia upon uninjured leaves. It was found that white mould lesions were produced similar to those resulting from infection with phragmospores from sporodochia on living leaves (Table II, nos. 1-6; Pl. I, fig. 5). Microscopic examination showed that conidia on all the lesions were of the *Ramularia* (phragmospore and amerspore) type. Fourteen reisolations from successful inoculations were made and all gave cultures typical of *R. Vallisumbrosae*. One of these cultures reisolated from pot no. 2 (Table II) was reinoculated successfully in the following season (see Table II, no. 12). In this series of inoculations, which were made with conidia taken direct from the fungal fructifications on natural lesions, thirty-one out of forty-two attempts were successful in producing typical white mould lesions bearing conidia of the

Ramularia type. Twenty-six of these infections were made with scolecospores produced from overwintered sclerotia and five with phragmospores and amerospores from leaf-spot lesions on a green leaf. While not in itself conclusive this series of inoculations provides further evidence that the scolecospores from sclerotia are part of the life cycle of *R. Vallisumbrosae*. This conclusion is confirmed by the three further series of experimental inoculations performed (except where indicated) with cultures started from single conidia of different types (Table II, nos. 9-26).

Table II. *Inoculation of Narcissus leaves with Ramularia Vallisumbrosae*

Pot no.	Nature of inoculum	Inoculated		Controls		Notes
		Sound	Infected	Sound	Infected	
A. Plants of "Golden Spur" ^a under bell-jars, inoculated 28 February 1936						
1-5	Scolecospores direct from sclerotia	34	14	112	1	14 reisolutions all <i>R. Vallisumbrosae</i> s.r.*
6	Phragmospores direct from sclerotia	8	5	13	0	
7-8	No inoculation	0	0	28	0	
B. Plants of "Golden Spur" under bell-jars, inoculated 25 April 1936						
9-10	1 α -scolecospore culture from sclerotia	6	6	13	0	
C. Plants of "Golden Spur", inoculated leaves protected test-tubes, 20 April 1937						
11	1 α -scolecospore culture from sporodochium	4	2	9	0	s.*
12	1 culture reisolated from lesions on pot no. 2 above	2	2	20	0	s.r.
13-18	6 1-scolecospore cultures from sclerotia	17	11	89	0	s.r.
19	1 1-amerospore culture from sclerotium	2	2	15	0	s.r.
20-23	3 1-scolecospore cultures from sporodochia	16	14	57	0	s.r.c.
24	1 1-phragmospore culture from sporodochium	4	4	12	0	s.r.
25	1 1-amerospore culture from sporodochium	2	1	10	0	s.r.
D. Plants of "Grandis" under bell-jars, inoculated 12 May 1938						
26	1 1-phragmospore culture from sporodochium	10	3	10	0	1 leaf had eel-worm
		105	64	388	1	

* Microscopic examination of lesions showed: s.=sclerotia; r.=phragmospores; c.=scolecospores.

The two cultures, nos. 11 and 12, referred to in Table II, were not started from single spores but from mass inocula; these were used for special reasons. In no. 11 was used the first strain isolated during the studies here recorded, from a leaf of Golden Spur collected in

Scilly in January 1935. After twenty-six months in culture the fungus had undergone certain changes, particularly in producing an even felty layer of aerial mycelium. It was included in the series to see whether it was still pathogenic. The result was that two out of the four leaves inoculated with it became infected, and the resulting lesions produced numerous characteristic sclerotia but no conidia. In no. 12 a strain reisolated from no. 2 was used and successfully reinoculated.

These inoculations show that white mould lesions bearing phragmospores of the *Ramularia* type, and sometimes also scolecospores, amerospores and sclerotia, are produced as a result of inoculating healthy *Narcissus* leaves with single spore cultures derived from any of the conidial types indicated in Table III, and it therefore concluded that they all represent conidial forms of *Ramularia Vallisumbrosae*.

Table III. *Summary of inoculations with Ramularia Vallisumbrosae*

Source and type of conidia	No. of single spore cultures tested
From sporodochia:	
Scolecospores	4
Phragmospores	3
Amerospores	1
From sclerotia:	
Scolecospores	7
Phragmospores	1
Amerospores	0

II. TAXONOMY

The evidence in the preceding sections indicates that *Ramularia Vallisumbrosae* Cav. and *Cercosporaella Narcissi* Boud. are probably names for the same fungus. It is true that the scolecospores of *C. Narcissi* ($50-130 \times 4-5 \mu$), are larger than those observed on white mould lesions during the present studies ($40-95 \times 2.5-3.5 \mu$), but it is clear that *Ramularia Vallisumbrosae* is subject to wide variation and it may be that Boudier's *Cercosporaella Narcissi* was founded on an exceptionally well developed specimen of *Ramularia Vallisumbrosae*. The specific epithet *Vallisumbrosae* antedates *Narcissi* by two years, so that if the two names ultimately prove synonymous, Cavara's name should be retained.

According to the International Rules for Botanical Nomenclature the names of genera of the *Fungi imperfecti* have only a temporary value, and it would not appear necessary for a *Ramularia*, if found to produce scolecospores, to be transferred to the genus *Cercosporaella*. Should it be thought desirable to name the organism according to the most highly developed spore form it produces, the name would become *C. Vallisumbrosae* (Cav.) comb.nov. In an analogous case,

Plakidas (1937) changed the name of *Ramularia Rubi* (Winter) Wollenweber to *Cercosporella Rubi* (Winter) Plakidas. Here, however, it is not proposed to change the name of the fungus on *Narcissus* which has long been known as *Ramularia Vallisumbrosae* Cav.

The classification of the fungus among the *Fungi imperfecti* presents certain difficulties because it cuts across some of the usual groupings. *Ramularia* and *Cercosporella* in Saccardo's classification are placed among the Mucedinaceae. Wollenweber (1913) regarded certain species of *Ramularia* as allied to *Fusarium* in the Tuberculariaceae, but later he placed some of them in his genus *Cylindrocarpon*. On its host plant *Ramularia Vallisumbrosae* certainly has the habit of one of the Tuberculariaceae. Soon after a portion of the leaf is invaded the mycelium begins to form plectenchymatous masses, and these organs must be regarded as fundamental in the economy of the fungus. The conidia are produced in sporodochia with compact plectenchymatous bases which may or may not be covered with a black sclerotic rind and the fungus appears to belong properly to the Tuberculariaceae. The question has been discussed by Mason (1937, pp. 78-81) who concludes that in the analogous genera *Fusarium* and *Cercospora* the presence or absence of a fungus stroma beneath the macroconidiophores is not a valid generic character. It may be noted in support of this conclusion that Istvánffi (1905) described and illustrated a mode of origin of fructifications of *Botrytis cinerea* on the vine in which the conidiophores arose in a group from a pseudoparenchymatous cushion, that is, in sporodochia, yet *B. cinerea* is not usually suspected of affinity with the Tuberculariaceae. The current classification of the *Fungi imperfecti* also fails to provide for conidia of either the *Ramularia* or *Cercosporella* type when produced from black resting sclerotia.

According to Mason's terminology (1937, pp. 85-86) all the conidia described in this paper are variations of *dry macroconidia*. No slime spore form has yet been linked with the life cycle, though it would be concordant with the studies of Klebahn (1918), Killian (1923, 1926) and others if these should one day be found. Mason (1937, p. 83) says: "*Ramularia* has slimy micro- and dry macroconidia borne on an entirely different mycelium from one another." In this connexion it is worth noting that there are two little-known imperfect fungi described from *Narcissus* foliage which appear to have slime spores: *Septoria Narcissi* Passerini, and *Phyllosticta narcissicola* von Keissler. Neither of these has yet been recorded in the British Isles, but in a letter to me Mr W. C. Moore says: "A pycnidial fungus was found in close association with the *Ramularia* in material received from the Isles of Scilly late in May 1926. Transverse sections of infected leaves revealed almost wholly submerged pycnidia mixed with the larger, partially sunken black bodies and with conidiophores

and conidia of the *Ramularia*. The pycnidia were spherical or nearly so, 55–75 μ in diameter, with a very short beak, and contained numerous, minute, single celled spores measuring approximately $3 \times 1 \mu$. Single spores transferred to malt agar slopes gave no growth, but *Ramularia* spores and sclerotia were produced in abundance when two pycnidia containing pycnosporos were carefully transferred to agar slants, although the possibility of *Ramularia* spores having been present on the pycnidia cannot be excluded. The pycnidia were quite distinct from those of *Phyllosticta Narcissi* Aderh. (= *Stagonospora Curtisii*) but corresponded to the description of *Phyllosticta narcissicola* von Keissler, which was recorded on *Narcissus poeticus* in Austria by von Keissler in 1916.”

Similar pycnidia containing minute phialospores have several times been found by me in sections among true sclerotia of *Ramularia Vallisumbrosae*. It may be that further investigation will reveal both perithecia and pycnidia of this fungus.

12. INTERPRETATION

That *Ramularia Vallisumbrosae*, like Killian's species of *Ramularia*, is a somewhat specialized facultative parasite is shown by: (1) its restriction so far as is known to a single host genus; (2) its ability to infect young uninjured leaves in full vigour; and (3) the adaptation of its life cycle to that of its host plant.

The thallus readily produces stromata which remain hyaline as sporodochia in the younger parts of the lesion, but become sclerotic on dead leaves and in culture.

The nature of the sclerotium is not at once obvious. It might be interpreted as merely a sporodochium which becomes sclerosed with the onset of unfavourable conditions and sporulates when the environment is suitable. However, while most of the sporodochia arise immediately beneath the cuticle, the sclerotia usually begin within an epidermal cell. The sclerotium also differs from the sporodochium in that it is clearly differentiated internally.

Another possibility to be considered is that the sclerotium is a peculiar organ transitional between a sporodochium and a pycnidium or perithecium. But this resemblance to the pycnidium is due only to a similarity in shape, while the conidia belong to different types. Unlike the conidia of *Septoria*, which are slimy spores, all the conidia of *Ramularia Vallisumbrosae* described in this paper are dry terminal spores. The genus *Phleospora* has fruit bodies which are apparently intermediate between sporodochia and pycnidia, but in them some of the host tissue shares in the formation of the stroma. No such inclusion of host tissue has been noted in *Ramularia Vallisumbrosae*.

The sclerotium may be compared with the spore-bearing organs

of *Cercoseptoria* Petrak (1925) (*Septoriopsis* Stevens & Dalbey (1919), non Fragoso & Paul, nec von Höhnelt) in which conidia of the *Cercospora* type are produced on tubercles of solid, nearly spherical masses produced subepidermally and later becoming erumpent. It is said that superficially and even in section they look much like the pycnidia of *Septoria*. The stromata of *Cercoseptoria* are apparently not covered by a sclerotic wall and they sporulate on living leaves. The sclerotia of *Ramularia Vallisumbrosae* differ in that the wall is composed of resistant carbonaceous cells, and in that they function after a resting period. The "intermediate bodies" of *R. Vallisumbrosae*, described on p. 35, which are regarded as transitional between sporodochia and sclerotia are, however, strikingly similar to the tubercles of *Cercoseptoria*, and this supports Overholts's (1938) statement that the genus "*Cercoseptoria* seems almost superfluous".

Reddick (1911) gave the name *pyncosclerotia* to structures in *Guignardia Bidwellii* (Ellis) Viala & Ravaz, which may develop after overwintering into either pycnidia or perithecia, and it is possible that these bodies are homologous with the sclerotia of *Ramularia Vallisumbrosae*. However, he never found any well-developed conidia borne on the tops of pyncosclerotia.

The studies of Klebahn (1918), Killian (1923, 1926, 1928), Laibach (1921), and Higgins (1920, 1929, 1936) have demonstrated the diversity of conidial forms associated with the genus *Mycosphaerella*.

Klebahn (1918) subdivided the genus *Mycosphaerella*, according to the type of conidial fructification formed, into: (1) *Septorisphaerella* with *Septoria* or *Phleospora* conidia; (2) *Ramularisphaerella* with *Ramularia* conidia; and (3) *Cercosphaerella* with *Cercospora* conidia. His description of *Mycosphaerella Hieracii* which has as a conidial stage *Ramularia Hieracii*, is interesting because of the occurrence of perithecia and micropycnidia, and also of sclerotia which sprout at the neck and produce *Ramularia* conidia. Klebahn illustrates a sclerotium of *Mycosphaerella Hieracii* which sprouted in moist conditions and formed conidia, in a manner quite comparable with *Ramularia Vallisumbrosae*, e.g. Text-fig. 30, but it appears from Klebahn's figure that the conidiophores of *Mycosphaerella Hieracii* arise from the top of the neck of the sclerotium, and not from the sides of a cavity in the neck as they do in the *Narcissus* fungus. A condition similar to *Mycosphaerella Hieracii* was described in *Ramularia Knautiae* by Laibach (1921).

Nannfeldt (1932) concluded that the imperfect forms in the genus *Mycosphaerella* belong to three principal types: (1) pycnidia with long thread-like conidia, such as *Septoria*, *Phleospora*, etc.; (2) pycnidia with bacterioid conidia, such as *Asteromella*, *Stictochorella*, etc.; and (3) hyphomycetous forms such as *Ovularia*, *Cercospora*, etc. It may be remarked that although many species of *Ramularia* and

Cercospora are known to have *Mycosphaerella* as a perfect stage, there are few records of perithecial stages belonging to *Cercospora*.

A condition very similar to that found in *Ramularia Vallisumbrosae* has been described by Killian (1923, 1926) who showed that members of the genus *Ramularia* are adapted to overwinter according to the habit of their host plant. He pointed out (1923) that the species of *Ramularia* studied by him show in over-wintering all transitions between perithecia, mixtures of perithecia and sclerotia, sclerotia alone and in *R. Geranii-pusilli* conidia alone on living leaves. Several other species investigated by Killian (1928) overwinter by resting sclerotia which germinate in the spring with the production of a crop of conidia on their apical part. In particular *R. Sambucina-racemosi* is figured, with a mass of polyhedral cells filling the sclerotium, but with elongated cells in the neck, a condition very like that found in *R. Vallisumbrosae*.

The interpretation adopted in this study is that *R. Vallisumbrosae* is probably the conidial stage of an as yet unknown member of the *Mycosphaerellaceae*. It is supposed that the sclerotia laid down in May are really the fundamentals of perithecia, which, for some reason, whether internal or external, take a different course in development and become sclerotic. After a dormant period the "meristematic" plug in the neck of the sclerotium behaves as a sporodochium. The occurrence in early stages of an organ resembling a trichogyne suggests that perithecia may one day be observed.

13. SUMMARY

Ramularia Vallisumbrosae Cav., the parasite causing the *Narcissus* white mould disease, appears regularly in the south-west of England. *Cercosporaella Narcissi* Boud. is probably a synonym.

On the living leaf the fungus produces conidia from subcuticular sporodochia. The conidia are typically scolecospores, but phragmospores and amerospores are also formed. The fungus aestivates by means of sclerotia which arise within the epidermal cells of the host leaves, but apparently the bulb is never infected. The sclerotia are differentiated into a body of polyhedral fat-storage cells and a neck of small cells rich in protoplasm, both surrounded by a carbonaceous rind. Under conditions in the south-west of England the sclerotia on old leaves lying on the soil produce conidia in January and February, and these serve to infect the new leaves after they come through the ground. The conidiophores line a depression in the neck of the sclerotium. The conidia are normally hyaline scolecospores but phragmospores and amerospores also occur.

Single spore isolations from the various forms of conidia both from sporodochia on living leaves and from sclerotia on over-summered leaves have given similar cultures. Inoculations with a number of

these cultures on to healthy *Narcissus* leaves have reproduced the white mould disease, and *Ramularia Vallisumbrosae* has been re-isolated from lesions so produced. These isolations and inoculations show that the various forms of conidia from sporodochia and sclerotia are part of the life cycle of the one fungus, *Ramularia Vallisumbrosae*.

The spore characters warrant the classification of the fungus either in *Cercospora* or *Ramularia*. In this paper it is preferred to retain the original name *R. Vallisumbrosae* Cav. If emphasis were placed on the mode of sporulation of the sclerotia a student might search for the name in vain among various genera. Like other fungi *R. Vallisumbrosae* shows variation in response to external and internal conditions. Some of the morphological features observed do not appear to have been described previously for any fungus, among these may be mentioned: (1) the differentiation of the sclerotium into a sterile fat-storing "body", and a sporogenous "neck"; and (2) the origin of the conidiophores from a depression in the "neck".

The sclerotia are considered to be developed from fundaments of an as yet undiscovered perithecial stage of the *Mycosphaerella* type.

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EXPLANATION OF PLATE I

Figs. 1, 2. Transverse section of leaf of *Narcissus poeticus ornatus maximus*, Isles of Scilly, April 1938. Stained eosin, mounted balsam. $\times 620$.

Fig. 1. Shows fundament of sporodochium which has ruptured cuticle and pushed down the outer cellulose wall of epidermis.

Fig. 2. Shows fundament of sclerotium in lumen of epidermal cell, pushing apart the inner and outer walls of epidermis.

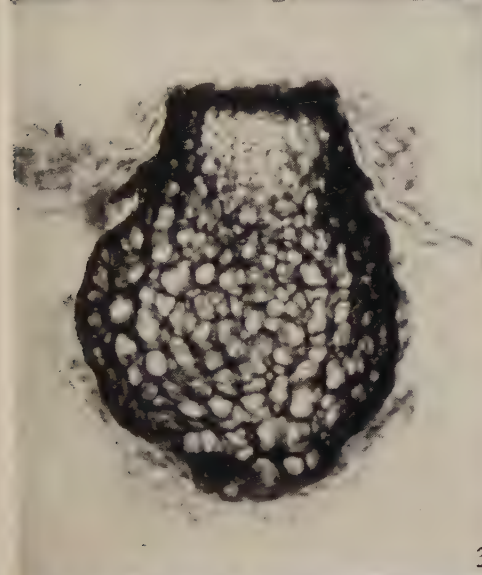
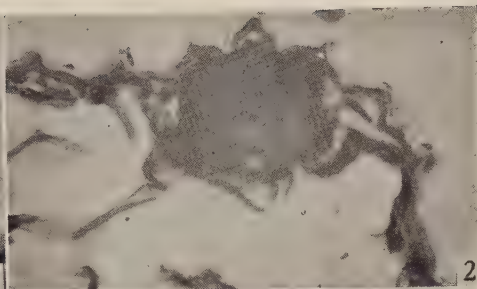
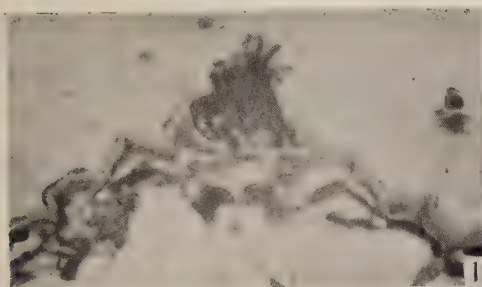
Fig. 3. Vertical section. Sclerotium in resting stage, October. Unstained, in glycerine jelly. $\times 620$.

Fig. 4. Surface view of leaf of *Narcissus* Golden Spur bearing sclerotia, aestivated at Penzance, sprouted in laboratory after three days' incubation in moist dish. $\times 55$.

Fig. 5. White mould lesions on leaf tips of *Narcissus* Golden Spur following inoculation with scolecospores taken direct from aestivated sclerotia (Table II, nos. 1-5). $\times 2$.

Fig. 6. Scolecospores produced from sclerotium, sprouted January 1936, on surface of soil, exposed at St Mary's, Isles of Scilly. Teased preparation, unstained. $\times 620$.

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ON AN ASCOMYCETOUS FUNGUS ATTACKING *RICCIA HIMALAYENSIS* ST.*

BY K. S. SRINIVASAN, B.Sc. (HONS.)
(University Botany Research Laboratory Madras)

(With Plate II and 14 Text-figures.)

INTRODUCTION

WHILE working on *Riccia himalayensis* St., I came across a few specimens which were attacked by an ascomycetous fungus. Kashyap (1929), in his account of the *Liverworts of the Western Himalayas and the Panjab Plains* (1929-32), recorded the fungus as occurring in this liverwort, but he did not mention the locality. In 1922, Prof. M. O. P. Iyengar sent him some material of this *Riccia* from Madras. This probably contained the fungus that he mentioned.

Kashyap's account of the fungus is extremely brief, and runs: "Some plants were once found attacked by an ascomycetous fungus which formed sunken perithecia with necks projecting on the dorsal surface. These could easily be mistaken for capsules. The perithecia were full of asci each containing two celled spores. This is the only liverwort, so far as I know, found attacked by an ascomycetous fungus bearing perithecia." As *R. himalayensis* St. is very common in Madras during the monsoon seasons, I undertook to study in detail the life history of this fungus.†

There are several previous records of liverworts being attacked by fungi. The majority of them belong to the Jungermanniales. But the records include a few members of the Marchantiales also, such as *Marchantia* (Kny, 1879; Golenkin, 1902), *Lunularia* (Kny, 1879), *Preissia* (Golenkin, 1902), *Targionia* (Golenkin, 1902), *Cyatodium* (Lang, 1905), *Plagiochasma* (Golenkin, 1902), *Ricciocaropus* (Garber, 1904) and *Riccia* (Duthie & Garside, 1936).

MATERIAL AND METHODS

The present investigation was carried out entirely by means of microtome preparations of field material. No cultural methods were employed. A fairly large collection of the living material of the

* This paper is one of three for which the Degree of Master of Science was awarded by the University of Madras.

† The fungus has now been described at the British Museum (Natural History) as *Phaerosphaerella Riccia* F. L. Steph.

Riccia was made and the obviously infected plants were separated with the aid of a Greenough stereoscopic dissecting microscope. Infected plants could be detected only after the fungus had developed either the spermogonium or the perithecium which generally appeared as dark specks on the dorsal surface of the liverwort. Plants with the earlier stage of infection could not be detected with the aid of the dissecting microscope, and sections had to be examined carefully under higher powers for these stages. The plants were thoroughly washed free of the soil particles and then fixed in strong chromo-acetic solution with 1 % osmic acid, Flemming's strong solution and Allen Bouin's P.F. A₃ mixture. Of these the P.F. A₃ mixture gave good results for the fungus. Microtome sections were cut ranging from 7 to 20 μ in thickness, stained in Heidenhain's iron alum haematoxylin and mounted in neutral canada balsam.

MYCELIUM

The vegetative hyphae are septate and much branched. The cells are more or less uniform in diameter, and usually each has one nucleus but occasionally two or three (Text-fig. 1). The infection starts in the lower epidermis of the host plant, and then proceeds upwards. No mycelium, however, could be found in any of the rhizoids, though many of the lower epidermal cells contained fungal hyphae. It is probable therefore that the infection comes from the soil and the fungus enters the liverwort, not through the rhizoids, but through the lowermost epidermal layer. During the first stage of its growth, the fungus ramifies through the ventral compact layer of the liverwort (Pl. II, fig. 5), and the assimilating filaments of the liverwort are not attacked until the formation of the perithecium or the spermogonium. The mycelium is intracellular (Text-fig. 4) and occupies the whole of the inside of the host cells, and uses up their protoplasmic contents. The fungus is somewhat localized in its attack and the infected part of the thallus appears as a definite and well-marked area.

Cavers (1903) and Nicolas (1932), while reviewing the work of previous authors, state that in some species only the gametophyte portion is attacked by the fungus, in others only the sporophyte portion and in others again, however, both the gametophyte and the

Legend to Text-figs. 1-14

Text-fig. 1, Vegetative mycelium, $\times 540$. Text-fig. 2, Sterigmata, $\times 1040$. Text-fig. 3, Spermogonium in surface view, $\times 300$. Text-fig. 4, Haustoria, $\times 540$. Text-figs. 5-9, Early stages in the formation of perithecium, 5, 6, $\times 540$, 7, 9, $\times 1040$. Text-fig. 10 a, b, c, Young proascus in three different foci, $\times 1040$. Text-fig. 11, Section of perithecium, $\times 180$. Text-fig. 12, Ascus with fusion nucleus, $\times 540$. Text-fig. 13, Later stage, $\times 540$. Text-fig. 14, Mature ascus with eight septate spores, $\times 1040$.



Text-figs. 1-14.

sporophyte portions are attacked. They express the opinion that where the fungus is found inside the sporogonium the relation between the fungus and the host is one of parasitism, while where the fungus is confined to the gametophyte portion, the association may be considered as symbiosis. In *Riccia himalayensis* only the gametophyte portion is attacked by the fungus. A number of sporophyte-bearing plants attacked by the fungus was examined carefully, but in none of them was the sporophyte portion infected. Following the opinion of Cavers (1903) and Nicolas (1932), the association of *Riccia himalayensis* and the fungus is inferred to be one of symbiosis, but I feel that before deciding whether the relation is one of symbiosis or parasitism, experimental work is necessary.

SPERMOGONIUM

Numerous spermogonia (Text-fig. 3) are formed in the upper part of the liverwort thallus. Though they appear to be imbedded among the assimilating plates of the liverwort, they are really situated on the surface region of the compact ventral tissue of the thallus, in the same manner as the assimilating plates or filaments (Pl. II, fig. 5). The mature spermogonium is pear-shaped, prolonged into a beak at the apex, and possesses a well-developed ostiole. The wall of the spermogonium is made up of about six layers of very much elongated pseudoparenchymatous cells which are thick-walled and brown. In the very young spermogonium the inner portion consists of a mass of loose filaments running parallel to the wall. In this central region, a number of radially arranged filaments (Text-fig. 2) (sterigmata) arise, with their free ends converging towards the centre. The filaments nearer the upper part, however, are directed more towards the ostiole. These sterigmata are rich in contents and take up a deep stain. Small elliptical spermatia are abstricted from their free ends. The entire cavity of the mature spermogonium is filled with these spermatia. The spermatium possesses a single nucleus which very nearly fills the entire cell cavity.

THE PERITHECIUM

Many perithecia in various stages of development were found on the thallus. The earliest indication of perithecium formation is the differentiation of certain hyphae from the surrounding ones (Text-figs. 5, 6, 7). Their cells are much larger and have very rich contents and a single prominent nucleus. These hyphae soon afterwards form branches, which, unlike ordinary branches, are very short and stout. Some of the lateral branches remain quite short, while others elongate a little and form secondary branches. Some of the branches grow

into a somewhat coiled structure (Text-fig. 8), on which a number of short lateral growths are formed, but the details of these lateral branches could not be followed in the sections. Sections of these portions at a slightly later stage showed a number of large multinucleate cells in the centre with their nuclei in pairs (Text-fig. 9). These large multinucleate cells evidently represent oogonia formed on the coiled branches. How the multinucleate condition arose could not be followed. No structure suggesting an antheridium could be found at any stage in the development of the perithecium. The vegetative hyphae surrounding these multinucleate oogonial cells become somewhat more compact and form a close investment round it. At this stage the developing perithecium appears somewhat globose, with the oogonial cells situated in the centre. The oogonial cells grow still further in size and in number, while their protoplasm becomes more and more vacuolated. At this stage a large number of paraphyses begin to develop from the base of the perithecium upwards, and soon occupy almost the whole of the space inside the perithecium. During the development of these paraphyses, the oogonial cells get gradually pushed down at the bottom of the perithecium, and soon afterwards begin to degenerate. The oogonial cells soon lose their sharp outline and finally become a more or less granular mass with the cytoplasm and the nuclei in various stages of disintegration. At a later stage, an intricate mass of hyphae with fairly rich contents is seen at the base of the perithecium passing through the degenerating granular mass of oogonial cells. These are evidently ascogenous hyphae, as a number of young asci were found at their ends. Even after careful examination of sections of numerous developing perithecia, no connexion could be established between any of the degenerating oogonial cells and the ascogenous hyphae. The ascogenous hyphae appear to start from the vegetative hyphae at the base of the perithecium or immediately below it. In the mature perithecium numerous asci are formed and are arranged in parallel rows in between the paraphyses (Text-fig. 11). The earliest stages of ascus formation from the ends of ascogenous hyphae were rarely seen. Only a few filaments were found showing some early stages. During ascus formation the end of the ascogenous hyphae is seen bent into a hook-like structure (Pl. II, fig. 2). At the bent region in the proascus, occasionally, two nuclei could be seen (Text-figs. 10a, b, c). The fusion of these two nuclei was not observed, but at a slightly later stage, in the young ascus a single large nucleus is seen (Text-fig. 12). The large size of this single nucleus and its rich chromatin content suggest that it is formed by the fusion of the two nuclei observed in proascus.

The stages of nuclear division in the ascus were not found, the only next stage available being the ascus with eight nuclei, arranged in

alternate rows. Stages showing the contents of the ascus divided into eight equal parts with a single nucleus in each were also found (Text-fig. 13). The next stage observed was the ascus with eight septate spores (Text-fig. 14). The final division of the uninucleate protoplasts into the septate spores was not seen. During the formation of the ascospores, a small portion of the protoplasm is left unused at the base of the ascus. The ripe ascospores are yellowish brown, and measure 2.1 by 6μ .

The perithecium is globose with a short funnel-like ostiole (Pl. II, figs. 1, 3, 4) and projects above the dorsal surface of the thallus of the liverwort. Its wall is hard and tough and is made up of several layers of cells. As the perithecium matures, it gradually becomes yellowish brown and finally black. The mature perithecium measures about 190μ long and 175μ broad.

SCLEROTIA

A number of dark, more or less globose structures are seen scattered in the ventral tissue of the infected thallus (Pl. II, fig. 5). These structures consist of a central mass of closely packed fungal hyphae with rich contents surrounded by an envelope made up of a single layer of large and more or less empty cells with very thick and dark brown walls. They are probably perennating organs of the nature of sclerotia and evidently serve to infect new liverworts in the next rainy season.

CONCLUSION

Some of the very highly developed Pyrenomycetes (e.g. *Polystigma rubrum*, *Gnomonia erythrostoma*, *Nectria galligena*, *Ophiobolus graminis*, and the present fungus) possess spermogonia. The spermatia were presumably functional originally, but have lost their function in the course of evolution. The oogonia that are now formed represent merely vestigial structures. They are formed still, and persist for some time, but finally degenerate and disappear. The present fungus also shares in a very large measure the characteristics shown by these spermogonia-bearing Pyrenomycetes. When the original sexual structures have lost their function and degenerated, the work of forming the ascogenous hyphae and the asci was taken up by the ordinary vegetative hyphae in the neighbourhood of the sexual structures.

SUMMARY

1. An ascomycetous fungus attacking *Riccia himalayensis* in Madras is described in detail.
2. The mycelium is intracellular and is confined to the gametophyte portion of the liverwort.

3. Many spermogonia and perithecia are formed by the fungus, and their development is followed in detail.

4. In the spermogonium, numerous uninucleate spermatia are abstracted from the ends of the radially arranged sterigmata and escape outside. The spermatia are non-functional and the spermogonia are merely vestigial organs.

5. During the development of the perithecium, a branched hypha is formed bearing a number of oogonia, but no ascogenous hyphae are formed by the oogonia. Through the profuse development of the paraphyses the oogonial cells are pushed to the base of the perithecium and finally degenerate into a plasmodial mass. Ascogenous hyphae then start from the ordinary vegetative hyphae at the base of the perithecium and grow through the plasmodial mass upwards. Numerous asci are formed by the ascogenous hyphae. The fully developed asci contain eight uniseptate spores.

6. Numerous sclerotia are formed by the fungus in the thallus of the *Riccia*. The sclerotia are probably perennating structures which serve to infect new liverworts in the next rainy season.

The author wishes to express his indebtedness to Prof. M. O. P. Iyengar for his kind guidance and help in the preparation of this paper. He also wishes to express his gratitude to Prof. Dame Helen Gwynne-Vaughan for her kind criticism of the paper. My acknowledgements are also due to the authorities of the University of Madras for the award of a research scholarship, during the tenure of which the work described above was carried out.

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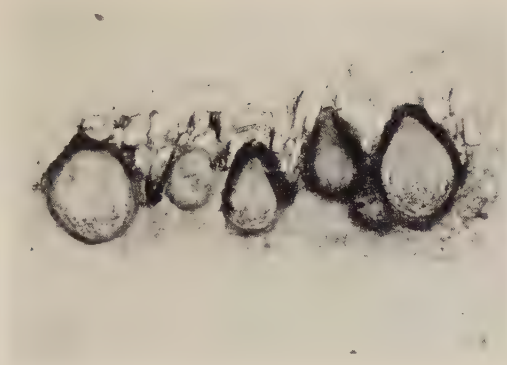
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EXPLANATION OF PLATE II

- Fig. 1. Series of perithecia in various stages of development. $\times 66$.
- Fig. 2. Crosier formation before the development of young ascus. $\times 530$.
- Fig. 3. Mature perithecium with asci showing eight double spores. $\times 530$.
- Fig. 4. Perithecium with asci and paraphyses. $\times 226$.
- Fig. 5. Thallus of the liverwort showing the fungus attacked area. Note the spermogonium formed dorsally and a number of dark sclerotia formed in the ventral compact layer of the thallus. $\times 80$.

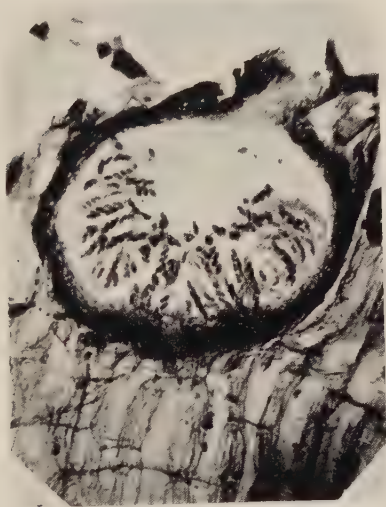
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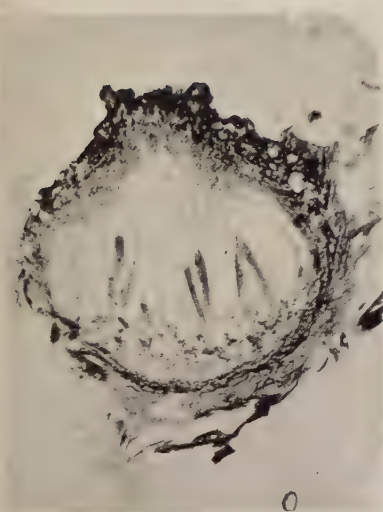
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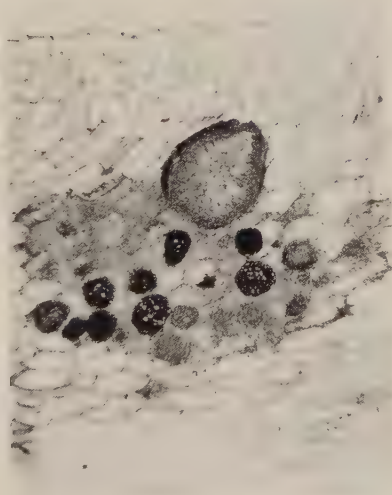
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A NEW SPECIES OF *PHAEOSPHAERELLA*

By FRANCES L. STEPHENS

SPECIMENS of the fungus described by Mr K. S. Srinivasan (p. 55) were submitted to the British Museum (Natural History) for identification. No fresh material was available and the description of the fungus had to be drawn up from a small amount of spirit material supplemented by microtome sections and observations made by Mr Srinivasan.

It has not been possible to match the fungus with any previously described.

Phaeosphaerella Ricciae n.sp.

Perithecia scattered or gregarious, brown, globose to pyriform, $200-500\mu$ diam., immersed, with a short neck, at least 50μ long, reaching to the surface of the host. Perithecial wall composed of 2-3 layers of pseudo-parenchymatous tissue. Asci cylindrical to clavate, tapering to the base, $80-120 \times 12-15\mu$, with oblique uniseriate or irregularly biseriate spores. Ascospores yellow to olivaceous, uniseptate, slightly constricted at the centre, fusiform, $16-22 \times 6-9\mu$; paraphyses or pseudoparaphyses sparse, hyaline and filamentous. Hyphae brown, septate, $5-6\mu$ wide, in the thallus and rhizoids.

Hab. in thallus of *Riccia himalayensis*, Madras, India.

Peritheciis sparsis vel laxe gregariis, immersis, brunneis, globosis vel ultra longis, erumpentibus; muris perithecialibus pseudoparenchymatis 2-3 seriatis; ascis cylindrico-clavatis, attenuatis ad basim, $80-120 \times 12-15\mu$, octosporis; sporis distichis vel oblique monostichis, olivaceis, fusiformibus, uniseptatis, ad septum leviter constrictis, $16-22 \times 6-9\mu$; paraphysibus vel pseudoparaphysibus sparsis, hyalinis, filiformibus; hyphis brunneis, septatis, $5-6\mu$ diam., in hospitis thallo rhizoideisque.

Hab. in thallus *Ricciae himalayensis*, Madras, India.

I have had difficulty in deciding upon the genus to which the species should be attributed. The well-marked neck of the perithecium suggests resemblance with *Rhynchostoma*, although in this genus the neck or beak is generally much longer, often as long as the perithecium is broad. Moreover, the members of this genus are generally found on twigs and woody tissues. In other respects, i.e. apart from the neck, the fungus appears to be a *Phaeosphaerella*, the sparse and ill-defined paraphyses indicating this genus rather than *Didymosphaeria*; consequently it is considered that it is best placed there.

It should be noted that although ripe ascospores are olivaceous, the colour washes out quite readily in spirit or lactophenol.

A number of Pyrenomycetes have been recorded on Hepatics. The nearest to Mr Srinivasan's fungus are (i) *Arcangelia Hepaticarum* Sacc. in *Riccia tumida* (Italy), which differs in having a shorter neck, hyaline spores and long hyphal-like hairs on the perithecia, (ii) *Phaeosphaerella Marchantiae* (Starb.) P. Henn. which has smaller spores and shorter neck, and (iii) *Lizonia emperigonia* (Aeursw.) de Not. which occurs on a moss, *Polytrichum commune*, not on a liverwort, and is quite superficial with a very short neck.

The pycnidia and sclerotia which also occur in the thallus of Mr Srinivasan's material of *Riccia himalayensis* are presumably organically connected with the above-described Pyrenomycete. None of the Sphaeropsideae so far recorded for Hepatics quite fit the one here described somewhat incompletely owing to lack of data. A similar pycnidium, though smaller, occurs in the specimens of *Phaeosphaerella Marchantiae* in Krieger's *Fungi saxonica*, No. 1924.

Pycnidia pyriform immersed up to 500μ diam., brown with a pseudoparenchymatous wall of several layers; pycnospores hyaline, $2 \times 1.5\mu$, the attachment to the conidiophores not observed, conidiophores lining the whole inner surface of the pycnidium. Sclerotia $25-120\mu$ diam., brown, globose to oval with large-meshed pseudoparenchymatous wall giving a reticulate appearance.

The differences between the measurements given above and by Mr Srinivasan are apparently due to his having been taken from immature and completely dehydrated specimens.

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STUDIES IN THE GENUS *USTULINA* WITH SPECIAL REFERENCE TO PARASITISM

IV. CONIDIA—GERMINATION AND INFECTION

By W. H. WILKINS

Mycology Laboratory, University Department of Botany, Oxford

(With 7 Text-figures)

I. INTRODUCTION

THIS paper is an investigation into the conditions which influence the germination of the conidia of *Ustulina vulgaris*, with the object of estimating the significance of the conidial stage as a probable means of infection of standing timber in temperate regions by that fungus.

The arrangement has been made to conform almost exactly with that of a previous paper by the author (1938) on infection by the spores of *Ustulina*. The present paper is to be regarded as complementary to the former one, and unnecessary repetition will be eliminated by frequent comparative reference to that paper.

II. PREVIOUS LITERATURE

Though the conidial stage is well known and often referred to from the taxonomic point of view, there appears to be no previous work on the germination of conidia in *U. vulgaris*. Several authors stress the fact that the conidial fructifications of *U. vulgaris* are almost identical with those of *U. zonata*, but most of the remarks on the conidia of the latter species are irrelevant in the present connexion.

III. MATERIAL

The conidial material used in the following experiments was collected during three successive seasons, 1934–6, from beech trees in various parts of the country, but mostly, by kind permission of the Duke of Marlborough and his agent Mr Sacré, from Blenheim Park, near Oxford. Fructifications were collected each week and kept at a laboratory temperature of about 15° C., in a damp atmosphere in glass dishes. Conidia are readily produced in culture, but in this work material collected from the natural habitat was always used. Fructifications were first seen about the beginning of April and were produced continuously, subject to weather conditions, as shown later, until September or October. Most of the experimental work was

done with conidia collected from April till the end of June. It is interesting to note that conidia collected from a fructification produced in late October gave a germination of 93 %.

IV. METHOD

As the type of experimental investigation is almost identical with that adopted for ascospores, the method employed was very similar. The hanging-drop technique as given previously was commonly used, except that the difficulty of separating conidia made it necessary to shake them up in a test-tube of the germination medium, since merely stirring them into a drop was not sufficient. The hanging drops were set up from the suspension.

The technical precautions mentioned in the previous paper were adhered to in this work. It might be well to repeat that experiments were done in triplicate and deductions based on the average of the three. That means that every "average percentage germination" or each point on a graph is based on a count of not less than a thousand conidia. In counting, it was usual to count at random six fields in each of four hanging drops. The problem of the age of the conidia was not so serious as in the spores and no correction, other than that mentioned later, was necessary.

V. THE FRUCTIFICATIONS

These are found growing on, or in close proximity to, the perithecial fructifications, and they are rarely found apart from them. They may form a comparatively dense covering over the substratum, as do the perithecial stromata, or they may appear irregularly scattered over the base of the beech trees or on beech stumps.

When growing freely on an exposed surface they tend to be more or less circular in outline, but when growing in crevices they become elongated and irregularly shaped. The young fructification is a minute white disc which increases in size by marginal growth. When about $\frac{1}{5}$ in. across, the centre becomes brownish and later, when about $\frac{1}{2}$ in. across, though this is very variable, the centre becomes grey. This indicates the presence of conidia. The mature fructification is a thin disc closely adpressed to the substratum to which it is attached at the centre. It may be up to 2 in. across, or, if elongated, several inches long. It is bluish grey with a white margin which disappears at maturity. Later the whole fructification becomes yellowish grey and is pulverulent with ripe conidia which are dispersed in this condition.

All this takes place in about a week or a fortnight. Under natural

conditions the conidial stromata subsequently gave rise to perithecial fructifications, but in the laboratory they inevitably became a putrid, grub-infested mass in about a week or ten days after collection.

VI. THE CONIDIA—SHAPE AND SIZE

The conidia are small, thin-walled and oval. In mass they are grey or greenish grey, but under the microscope they appear hyaline.

Conidia, in optimum conditions for germination, were measured. It was found impossible to measure them dry, as was done with spores, for when separated from the conidiophores they quickly shrank. They were therefore measured in distilled water under a cover-slip, with a $\frac{1}{12}$ in. oil immersion objective and micrometer eyepiece. The range of size and the lengths and breadths of 238 conidia are given in Table I.

Table I. *Measurements of conidia*

Length		Breadth	
Range	Average size	Range	Average size
6.0-9.0	7.31 $\pm$ 0.003	3.0-3.75	3.06 $\pm$ 0.0001

The variation in length is not very great, while in breadth it is remarkably small. As would be expected under these circumstances, the standard deviation calculated from the formula $\delta = \sqrt{\frac{\Sigma(u_x)^2}{n^2}}$ was significantly small. The deviation of a single individual is, for the length, ± 0.26 , and for the breadth ± 0.048 . The approximate size is therefore $7 \times 3 \mu$.

For purposes of comparison, sizes of conidia of *Ustulina* of temperate regions as found by other investigators are given below:

Tulasne	1863	6.5	$\times 3.5 \mu$
Winter	1887	6.5	$\times 3.5 \mu$
Traverso	1906	6.5	$\times 3.5 \mu$
Van Overeem	1924	6.0-8.0	$\times 2.8-3.5 \mu$
Miller	1930	5.0-6.5	$\times 2.0-3.0 \mu$

There is little variation between these measurements, but on the whole the conidia appear to be rather shorter and wider than in the present work. There is, however, no indication as to how the above conidia were measured, so direct comparison is impossible.

VII. DETERMINATION OF A FAVOURABLE GERMINATION MEDIUM

Conidia were germinated in hanging drops at 25° C. for forty-eight hours in solutions similar to those used for spores, and comparison between germination of spores and conidia is thus possible. The pH

of the solutions was determined before use. The percentage germination was calculated and the germ-tube lengths were measured. The results are shown in Table II. These results are given in full to illustrate the general method and to show the degree of consistency obtained when triplicating the experiments. Subsequent results will be given as total average percentage germination only.

Table II. *Germination in water and various wood extracts*

	pH	No. exam.	No. germ.	%	Av. %	Germ tubes
Distilled water	7.0	312 412 348	211 322 309	67.63 78.15 88.79	78.19	Straight and unbranched, 2 per conidium. Average length 10 μ
Rain water	7.1	457 432 461	265 203 270	57.99 47.00 58.57	54.52	Straight and unbranched, 1-2 each. Average length 4.7 μ
Tap water	7.2	370 376 352	16 15 8	4.32 3.98 2.27	3.52	Straight and unbranched, 1-2 each. Average length 4.8 μ
Distilled water extract of beech wood	6.8	327 346 264	267 272 202	81.65 78.61 76.52	78.92	Mostly straight, unbranched and 1 each. Average length 19.6 μ
Rain water extract of beech wood	6.9	400 264 360	349 222 259	87.25 84.09 71.94	81.09	Mostly straight, unbranched and 1 each. Average length 20.5 μ
Tap water extract of beech wood	7.0	360 302 236	352 285 219	97.22 94.37 92.80	94.79	Straight and unbranched, 1-2 each. Average length 22.5 μ
Tap water extract of bark	5.8	607 440 427	324 225 205	53.38 51.14 48.01	50.84	Irregular and branched, 1-3 each. Average length 4.1 μ
Natural rain water from base of tree	7.2	452 431 457	129 96 128	28.54 22.27 28.00	26.27	Straight and unbranched, 1-2 each. Average length 5.0 μ
Tap water extract of diseased wood (<i>Armillaria mellea</i>)	6.6	550 463 268	299 193 219	54.36 41.68 48.88	48.30	Straight and unbranched, 1-2 each. Average length 11.8 μ

From this table it will be seen that the best medium, from the standpoints of both percentage germination and growth of germ tubes, was tap water extract of beech wood. This agrees with the result of the comparable experiment on spores. In general, conidia germinate better than spores in distilled water, rain water, distilled water extract, rain water extract, tap water extract and diseased wood extract, and worse than spores in tap water (very bad indeed), tap water extract of bark and natural rain water. In a total count of about twelve thousand spores and conidia respectively in all the above media, the spores germinated on an average to about 40% while the conidia germinated to about 60%. Under similar conditions and in the same time, conidia germinate as quickly and to a rather higher percentage than do spores.

Subsequent experiments were done in tap water extract, partly because it gave almost maximum germination, and partly in order to facilitate comparison with spores.

VIII. GERMINATION

No detailed observations have been made on rate of germ-tube growth. According to the germination medium, they put out one or more germ-tubes. They have never shown more than five per conidium. When the tubes are single they are relatively long and

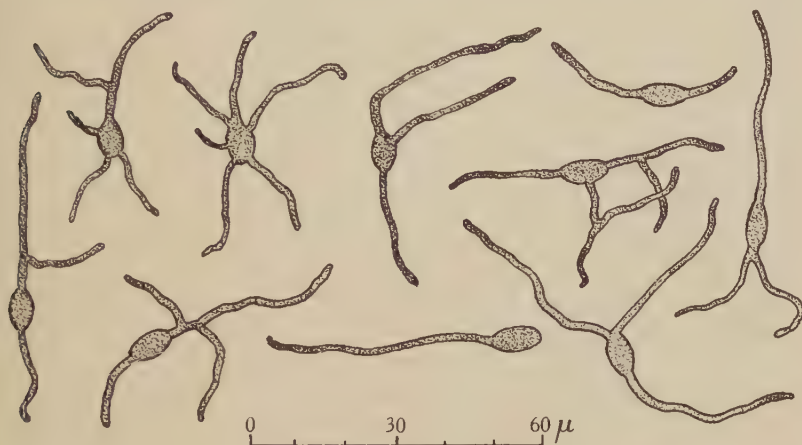


Fig. 1. Types of conidial germination.

when there are several they are correspondingly shorter. There is no apparent swelling up of the conidium before germination. Single germ-tubes usually, but not invariably, come out of one end of the conidium while two tubes usually grow out one from each end. The tubes, which are usually about 2μ in diameter, often branch freely. Illustrations of types of germination are shown in Fig. 1.

IX. FACTORS INFLUENCING GERMINATION

(a) *Germination in relation to age*

As previously stated, the age of conidia is a factor which can easily be allowed for. As long as conidial fructifications are being produced at all, there is little variation in percentage germination throughout the season. At each weekly collection during 1935 a control experiment in tap water extract was set up and it was found that the range of variation from week to week was between 75 and 87%. There is no falling off in germination capacity, as there was in the spores, due to age, dating from the beginning of the conidial season.

The "age" of the conidia dating from the initiation of each fructification expressed in terms of development of fructification and maturation of conidia had, however, to be taken into account. Very young fructifications were collected, brought into the laboratory and kept in a damp atmosphere. The fructifications are about two days in a white stage, two days in a brownish stage and two days in a grey stage before the conidia are mature, and no germination takes place before this mature stage.

Every twenty-four hours conidia were taken from the mature fructification and germinated for forty-eight hours at 25° C. The

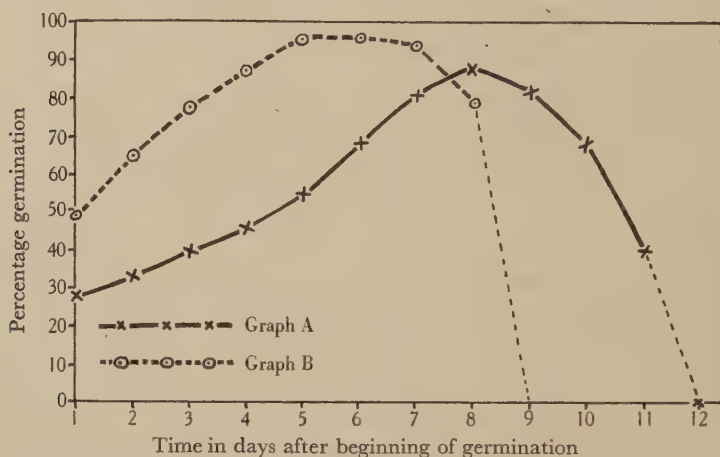


Fig. 2. Relation between "age" of conidia and germination capacity.

percentage germination of each set was calculated. This was continued till it was impossible to obtain conidia owing to the degeneration of the fructification. It was usually possible to carry this on for about ten to twelve days. This experiment was repeated several times with remarkably consistent results. The average of these results is shown in Fig. 2, Graph A.

Graph A shows that the optimum age for germination is from about six to ten days after the first appearance of the grey colour. This stage can be recognized by the superficial appearance of the fructification, which was dark or yellowish grey, the surface being flaky, becoming pulverulent. Conidia for experimental work were always chosen while in this stage, so that a percentage germination of from 65 to 85 could be expected, and with experience this experimental period could be restricted to the stage corresponding to the seventh to ninth days as shown in the graph, giving a constant percentage germination of 80. It was possible to collect fructifications

in a stage such that they could be used in optimum conditions almost at once, and hence prolonged keeping in the laboratory was avoided.

This developmental period varied with the environmental conditions. Graph B deals with this aspect and will be referred to later in the section on humidity, p. 79.

(b) *Temperature*

(i) *Determination of optimum temperature.*

General observations on the significance and limitations of the term "optimum temperature" have been made in the previous paper (1938, p. 65). Conidia were germinated in four different germination solutions, viz. rain water, tap water extract of beech wood, starch medium and Leonian's medium at the seven following temperatures: 5, 10, 15, 20, 25, 30 and 35° C. Percentage germination and germ-tube lengths were recorded. There was no germination at 5 or 35° C. At the other five temperatures germination took place in all the media, but was best in tap water extract and worst in rain water. When the percentage germination at 25° C. in rain water and tap water extract in this experiment is checked back with the percentage germination obtained in the same two media as given in Table II, it is found that the results are very consistent, as rain water gave 55.19 and 54.52 % and tap water extract gave 94.98 and 94.79 % respectively. The optimum temperature was always between 20 and 25° C. but the results are only recorded for germination in tap water extract.

Table III. *Determination of optimum temperature
in tap water extract of beech wood*

Temp. ° C. ...	10	15	20	25	30
Av. % germination	13.66	63.83	90.10	94.98	60.92
Av. tube length in μ	4.80	15.20	26.10	37.20	11.10

A comparison between germination of conidia and spores in relation to temperature is illustrated in Fig. 3.

These results bring out the following points:

(1) Whereas the optimum temperature for conidia is between 20 and 25° C. that for spores is higher, viz. 25–30° C.

(2) The percentage germination is higher in conidia in the same time period.

(3) Conidia germinate more rapidly at low temperatures, for they will germinate in forty-eight hours at 10° C., while a week at 10° C. was not sufficient to induce germination in spores, and whereas at 15° C. conidia give a percentage germination of about 65, spores give only a 35 % germination.

(4) There is a wider range of temperature toleration by conidia. This is significant in connexion with infection, for though as Fawcett (1921, p. 186) says, for many parasitic organisms it is probable that the temperature range within which serious infection of their hosts may occur naturally is comparatively narrow, nevertheless the wider the range of toleration the less probable the limitation of infection by the temperature factor.

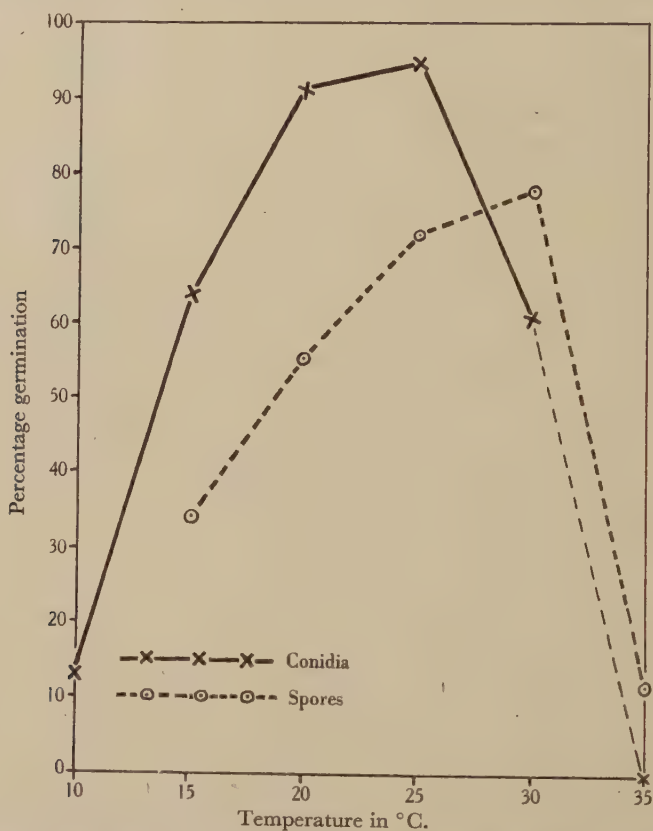


Fig. 3. Comparison between germination of conidia and spores in relation to optimum temperature.

(ii) *Effect of maintained temperature on germination.*

An experiment on the influence of maintained temperatures on the germination of conidia was performed in exactly the same way as in the spores, except that, here, there was no need to make any allowance for the age factor. Conidia were germinated at 10, 15, 20, 25, 30 and 35° C. The percentage germination was calculated at the end of

twelve, twenty-four and every subsequent twenty-four hours for a total period of 120 hours. There was no germination at 35° C. but the results of germination at the other temperatures are shown in Fig. 4.

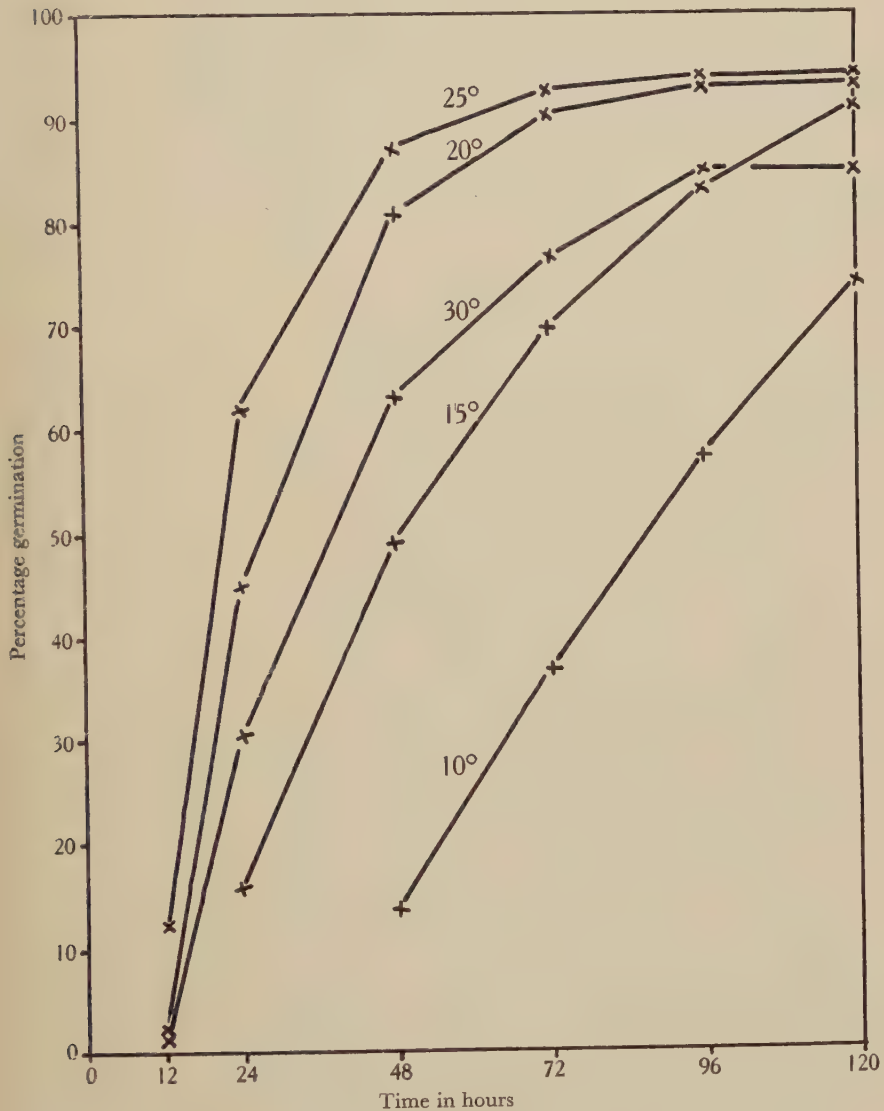


Fig. 4. Effect of maintained temperatures on germination.

These results are comparable with the similar graph of October spores as both conidia and spores were in optimum physiological condition, and both were germinated in similar media and examined at the same time intervals.

Comparison between the two graphs shows that there is a striking similarity. In the conidia, though the rise to almost maximum germination after forty-eight hours is apparent, it is reached slightly more slowly than in the spores. The outstanding difference between the two is the much more vigorous germination of conidia at low temperatures. Germination at 15° C. is extremely good, while germination at 10° C., which did not happen in the spores, would reach the maximum in about a week. On the other hand, germination is not quite so good at 30° C. and there was no germination at all at 35° C.

(iii) *Upper and lower temperature limits.*

It has already been determined that there is no germination at 35° on the upper and at 5° C. on the lower side of the optimum. The upper temperature limit for growth is therefore between 30 and 35° C. and the lower limit between 5 and 10° C. 35 and 5° C. have been taken as being the upper and lower temperature limits respectively. In order to find the upper and lower thermal death-points of conidia, experiments were carried out in the same medium and by the same method as that used for spores.

The lower thermal death-point. The experiment was set up using tap water extract. About forty ring cells were incubated at 0 and 5° C. respectively, and sets of five cells were transferred from each to optimum temperature (25° C.) after twelve hours, twenty-four hours and successive intervals of twenty-four hours up to 192 hours. In each the percentage germination was calculated after forty-eight hours at the optimum. A control set of five cells was incubated at 25° C. during the whole period, and the percentage germination was calculated after sixty hours and at successive intervals of twenty-four hours until the end of the experiment (see Fig. 5).

Conidia which had been at 0 and 5° C. throughout the experiment germinated when they had been transferred for a suitable period to optimum. Though there is no established standard definition as to thermal death-points in the conidia, it is obvious that neither 0 nor 5° C. can be considered as thermal death-points in relation to any reasonable time period.

There were no facilities for experimental work below 0° C., so that the lower thermal death-point could not be determined. The effect of low temperatures on conidial germination is interesting and confirmatory of the state of affairs which obtained in the spores. The

results are plotted graphically in Fig. 5, which for purposes of comparison also shows the graph of the effect of exposure to 0°C . on spores. When plotting the two graphs of conidia at 0 and 5°C . it was found that the points came so close together that they could be connected by one smoothed curve. This was therefore done, the 0°C . points being represented by $\odot$ and the 5°C . points by $\times$.

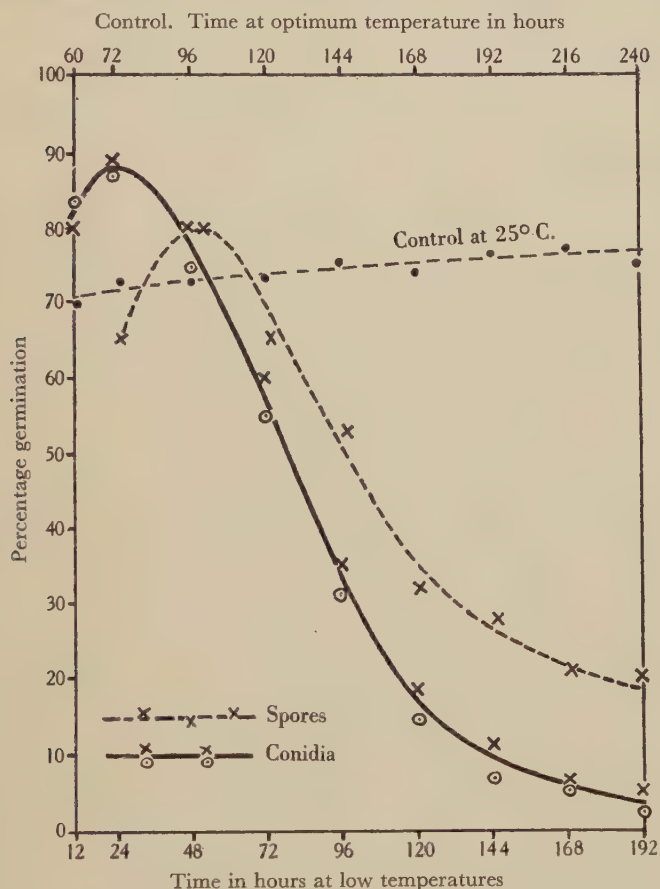


Fig. 5. Determination of lower temperature limits for germination.

It is evident that exposure to low temperatures has a stimulating effect on the germination of conidia. Moreover the stimulation appears to be of equal intensity, or at any rate produces equal response, at both the low temperatures tried. An exposure of twelve hours produces a percentage germination of about 80, that is, 10% higher than the control, while after twenty-four hours' exposure the

germination is about 90 %, which is about 20 % higher than the control. Beyond this period the germination falls off uniformly and steadily till it has almost reached 0 % at the end of the experimental period of 192 hours.

This conidial graph differs from the spore graph in several ways:

(1) In the conidia exposure to 0 and 5° C. produced relatively the same effect, whereas in the spores 0° produced a greater stimulus than 5° C.

(2) Response to the stimulus was more rapid in the conidia, as they reached their peak of 90 % in twenty-four hours whereas in the spores the maximum germination of 80 % was reached only after forty-eight hours.

(3) The fall off was more rapid and complete in the conidia, though with each type of spore there was a subsequent slowing up.

It seems reasonable to suggest that the differences here indicated may be due to the increased resistance of the spores. Though conidia are more tolerant of low temperatures for short periods, prolonged exposure decreases their germination capacity rapidly. Moreover, at the lower limit of their toleration, conidia are more sensitive to this inhibitory effect, 5° C. having as great an effect in this respect as 0° C.

The conidia are undoubtedly killed by continued exposure to a temperature as low as 5° C. This death curve of the conidia is reminiscent of the curve made by Henderson Smith (1923, p. 336) for the killing of spores of *Botrytis* by heat and an almost similar curve by the same author (1921, p. 31) on the killing of spores of *Botrytis* by phenol.

The upper thermal death-point. This was much more definitely determined. There was no germination at 35° C. so conidia were incubated at 35, 40, and, as a control, at 25° C. by the same experimental method as that used above, except that in the present experiments the time periods used were greatly reduced. After two hours' incubation at 40 and subsequent transference to 25° C. for forty-eight hours, there was no germination. After an exposure of one hour there was, rarely, up to 1 % germination, so that, on the whole, this can be considered, on the basis of the lower thermal death-point argument, rather beyond the upper thermal death-point. The results for germination at 35 and 25° C. are given in Table IV.

It will be seen that there is a rapid fall off in percentage germination after exposure to a temperature of 35° C. The control after approximately forty-eight hours at 25° C. gives a percentage germination of about 87, but the conidia which have been exposed for only one hour to 35° before their subsequent period at 25° C. for forty-eight hours show a percentage germination of only 30. After six hours' exposure to 35° C. there is no germination at all on subsequent

incubation at optimum, so that 35° C. can be established as the upper thermal death-point. Exposure to high temperatures does not produce stimulation of germination in the conidia, but, on the other hand, rapidly causes their death.

Table IV. *Determination of upper thermal death-point*

Experiment			Control	
Time in hr. at 35° C.	Time at 25° C.	Percentage germination	Time at 25° C.	Percentage germination
1	48	30.07	49	86.93
2	48	15.49	50	87.35
4	48	4.07	52	87.48
6	48	0.00	54	88.03

(iv) *Relation between temperature and probability of infection.*

This was estimated exactly as in the spores. The average weekly maximum and minimum outdoor temperatures were plotted, from Oxford records, for the six months April to September, together with similar average weekly temperatures taken in a beech wood.* Fig. 6 shows the relationship between range of outdoor temperatures and the possibility of conidial germination during the conidial season from April to September.

A comparison between Fig. 6 and the similar figure for spores (Wilkins, 1938, Fig. 10, p. 74) shows very significant differences. In the spores, the temperature range between optimum and the lower limit for germination fell almost entirely outside the range of temperature occurring during that period in nature, whereas in the conidia the temperature range for germination and the temperature obtaining in nature correspond very closely. This correspondence is brought about because, first, the range of temperature in which conidial germination is possible is slightly lower than in the spores and, secondly, the average out-of-doors temperature is higher during the conidial season than during the spore season. Though germination and hence infection by spores was most probably inhibited or depressed by the temperature factor, conidial germination seems to be favoured by this factor and, other conditions also being favourable, infection by conidia seems to be highly probable.

(c) *Humidity*

The effect of relative humidity on germination of conidia was ascertained by putting dried-off films of a suspension of conidia in tap water extract in ring cells containing solutions of different

* For the beech wood figures I am indebted to Mr G. M. Harris, B.A., B.Sc., a research student in this laboratory.

vapour pressure made up according to the method of Stevens (1916). The resulting relative percentage humidities were respectively 100, 90, 80, 70, 60 and 50. All the cells were incubated at 25° C. for forty-eight hours. A control with conidia in liquid tap water extract was set up at the same time and under the same conditions.

Germination took place only in 100 % relative humidity. Though the experiment was repeated four times, there was never any

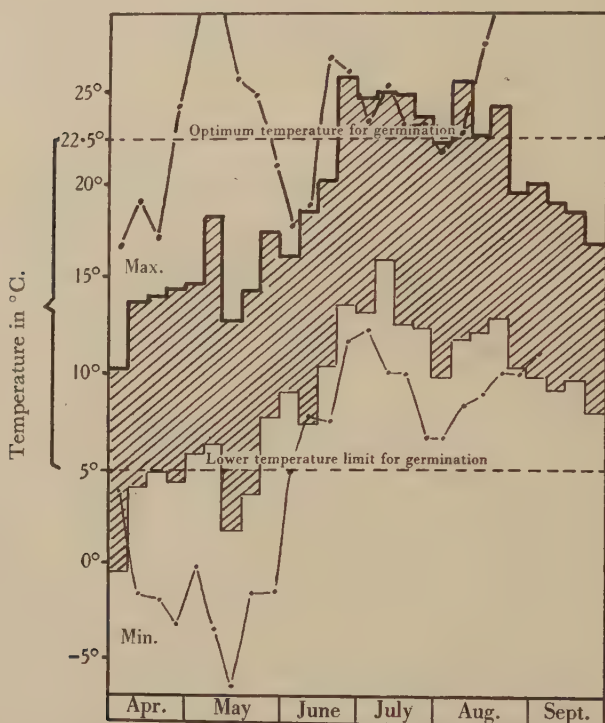


Fig. 6. Relation between out-of-door temperature range and temperature limits of germination.

germination at any of the other humidities after forty-eight hours, neither was any effect produced by prolonging the time of exposure beyond forty-eight hours. A comparison between percentage germination in a saturated atmosphere and in liquid extract showed that germination can take place in a saturated atmosphere to a percentage not exceeding 20, while in the liquid extract control there was a consistent germination percentage of about 80. In the spores, a saturated atmosphere failed to produce germination, a liquid medium being essential.

In connexion with germination of conidia, humidity is a most important factor. It was found that in nature, rainfall and relative humidity had a most marked effect on the production and development of conidial fructifications and the viability of the conidia themselves. Each time the weekly collection of conidia was made it was evident that the number of fructifications which had developed since the last collection depended entirely on the previous week's rainfall. In fact, it was no rain, no fructifications, much rain, abundant fructifications. A damp period with an average rainfall of about half an inch a week is satisfactory if the rainfall is distributed. This amount per week distributed so that some rain falls every day is much more effective in the production of fructifications than is one inch of rain which falls all on one day of the week.

Another important point is that fructifications which, though produced in a wet period, are subsequently exposed to dry weather produce conidia which either will not germinate at all or only with difficulty. Fructifications produced and developed in a continuously wet period, on the other hand, produce conidia which will give a percentage germination of about 90–100. In May and June of 1935 rainfall was low and conidial stromata developed slowly, as explained in section ix, p. 69, and as illustrated in Fig. 2, Graph A. In the same period of 1936, however, the average rainfall was comparatively high and hence the conditions for conidial development were more favourable. This resulted in a more rapid maturation of the conidia and a higher germination rate when they were at the optimum age for germination. This is shown in Fig. 2, Graph B (p. 70), where it will be seen that the optimum period for germination is not the 7th–9th day as in Graph A, but about the 5th–7th day. The maximum percentage germination also has risen from 85 to 95. In the second example the fall off is also quicker, there being no germination after nine days, as the fructifications have then disintegrated.

From the above it is clear that a well-distributed weekly average of not less than half an inch of rain is essential to produce optimum conditions for conidial production and development, and it is obvious that the conditions which favour the production of conidia are also those which are favourable for germination and infection.

(d) *The medium*

(i) *Nutrient aspect.*

The nutrient aspect of the germination medium was tested by the same detailed experiments as in the spores. A brief comparative summary of results is given here, but the tables are omitted. When conidia were germinated in the twelve nutrient media (cf. Wilkins, 1938, Table VIII) it was found that germination varied according

to the medium. The conidia germinated best in starch medium and oatmeal, both giving over 90 %. Leonian's was the best synthetic medium for conidial germination.

The "individual constituent" experiment (cf. Wilkins, 1938, Table X) usually gave high germination within the region of 70–80 %, the only unfavourable constituents being sodium nitrate giving 24 %, potassium chloride giving 47 %, tri-potassium phosphate giving 24 % and di-potassium acid phosphate giving 40 %.

When this experiment was repeated with the constituents on a molecular basis (cf. Wilkins, 1938, Table XI), the same result was obtained. In both the above experiments, the potassium phosphate salts showed increasing germination in relation to the increasing acidity of the salt.

	K_3PO_4	K_2HPO_4	KH_2PO_4
First experiment	23.9	40.1	61.1 %
Second experiment	32.7	46.7	76.8 %

The various permutations and combinations of the constituents of Leonian's medium (cf. Wilkins, 1938, Table XII) gave over 70 % germination throughout except with magnesium sulphate, when germination averaged 63 %.

It appears from the above that there is much similarity in the reactions of spores and conidia respectively to the composition of the germination media. It should be noted, however, that though the addition of certain substances to distilled water increases the germination to the maximum of both spores and conidia, it makes less difference to conidia than to spores, as conidial germination in distilled water was approximately 75 %, whereas that of spores was about 50 %.

(ii) *The effect of hydrogen-ion concentration.*

In order to test the influence of the pH of the germination medium, conidia were germinated in different media each of which was adjusted over a range of pH from 2 to 9. The adjustment was made by means of hydrochloric acid and sodium hydroxide. The media used were rain water, tap water extract, starch medium and Leonian's medium. The method adopted was that used for the spores, and the ring cells were incubated at 25° C. for forty-eight hours as usual. The results are shown in Fig. 7.

There was a distinct difference between germination in the two natural media, rain water and tap water extract, and germination in the two synthetic media. In the former the pH optimum was sharply defined at pH 6, with an almost symmetrical drop to pH 3 on the acid and pH 9 on the alkaline sides respectively. With the synthetic media, however, the optimum was less sharply defined, being from pH 5 to 6, and the range of high germination percentage

was greater. This bears out the statement by Webb (1921, p. 328) that "The effect of the hydrogen-ion concentration of the medium as influenced by certain types of nutrition, or the chemical composition of the nutrient medium, is shown...to be of extreme importance." In the synthetic media also, the percentage germination was rather higher, that in Leonian's reaching almost 100 at optimum

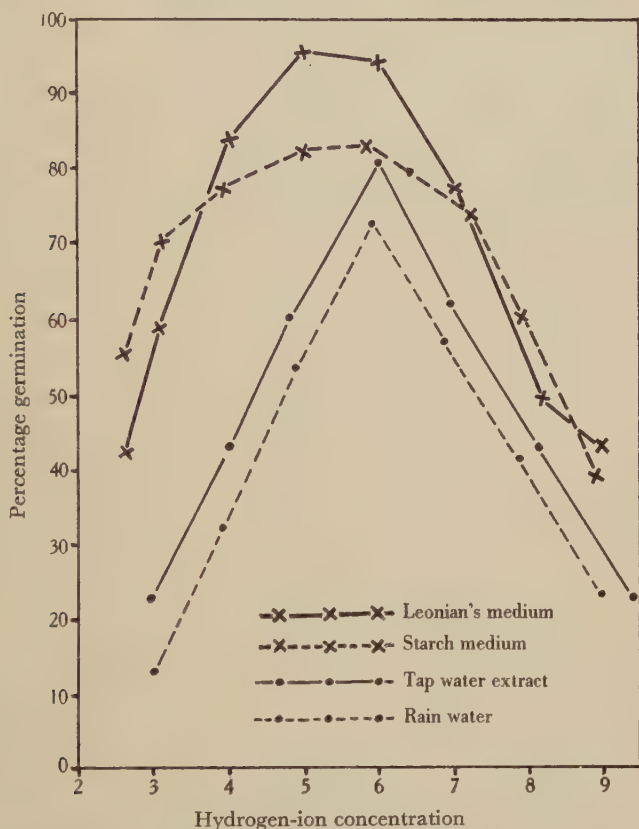


Fig. 7. Effect of pH on germination of conidia in four different media adjusted by hydrochloric acid and sodium hydroxide.

pH. There was no germination at pH 2 on the acid side, but the limit on the alkaline side was not determined, as it was beyond pH 9.

In comparison with spores, the optimum pH for conidia was less definitely at 6. In the synthetic media there was a slight tendency to incline to the acid side, as in both these the germination at pH 5 was equally good or better. Moreover, conidia germinated at pH 2.5, which spores did not, but whereas spores germinated between 50

and 70 % at pH 9, conidia rarely showed a germination above 40 % at this point.

In order to see whether the method of adjustment made any serious difference to the results, the experiment was repeated, the media now being adjusted with sulphuric acid and potassium hydroxide. The results did not differ essentially from those obtained when the media were adjusted with hydrochloric acid and sodium hydroxide.

X. INFECTION

(a) Germination of conidia in different timber extracts

Conidia were germinated in tap water extracts of the same seven kinds of wood as were used for spores. Extracts were made up from both green and seasoned wood, and the pH of each solution was tested. The percentage germination was calculated after incubation at 25° C. for forty-eight hours. The results are given in Table V.

Table V. Germination in different timber extracts

	Seasoned		Green	
	pH	Av. % germination	pH	Av. % germination
Ash	7.0	32.98	6.8	54.49
Beech	6.6	83.38	6.6	77.59
Elm	7.2	34.33	7.2	92.27
Horse-chestnut	7.0	64.45	7.2	48.49
Lime	6.9	42.87	7.2	94.91
Oak	4.6	0.0	5.1	62.38
Poplar	7.4	74.29	6.8	74.29

Comparison between the above table and Table XVI in the spore paper shows that conidial germination in these wood extracts is even more variable than that of spores. The extracts of green wood give much better germination, i.e. an average of 72 %, than those of seasoned wood, which gave only 42 %. The total average percentage germination for conidia is approximately twice that for spores. The best germination was in extracts of beech, elm, lime and poplar, and it is interesting to note that instances of decay by *Ustulina* have been found on all these trees, though the method of infection was not determined.

(b) Penetration of wood

Small blocks of the above seven timbers were sown with conidia on the transverse face, and incubated at the four temperatures, 15, 20, 25 and 30° C. Degree of penetration was measured at the end of seven days. In all, penetration was greatest at 25° C. (cf. Wilkins, 1938, Table XVIII), with the exception of oak, in which there was no penetration. The average degree of penetration at 25° C. is given in Table VI.

Table VI. *Penetration (in mm.) of different kinds of timber at 25° C.*

Ash	Beech	Elm	Horse- chestnut	Lime	Oak	Poplar
4.2	2.1	2.6	1.3	4.75	0.0	5.4

(c) *Inoculation experiments*

Twelve young lime trees and twelve young beech trees were inoculated with conidia and examined at the end of twelve months. As in the inoculation experiments with spores, infection occurred in all. Some additional trees were similarly inoculated and left standing for five years. At the end of this time the trees showed no external signs of disease, which may be due to the fact that young vigorously growing trees are able to withstand disease even if infection occurs, whereas older trees are less resistant, owing to the presence of dead heartwood.

XI. CONCLUSIONS

It appears that both the conidia and the spores of *Ustulina* can germinate on and infect living timber. The extent to which this infection takes place in nature and leads eventually to decay of living trees has not been determined. It has been found that *Ustulina* causes a disease of the heart-rot type in trees of temperate climates and there must obviously be some means of infection. It is assumed that such infection is brought about by spores or conidia, and the results of this and the previous paper suggest that infection by conidia is the more probable for the following reasons:

- (1) Conidia are lighter and more easily dispersed.
- (2) They are produced in greater abundance.
- (3) They germinate to a higher percentage in the same time.
- (4) They are produced in a "high-percentage germination" condition over a longer period.
- (5) Germination is more rapid in water extracts of green timber.
- (6) They have a wider range of temperature within which germination can take place, particularly on the suboptimal side.
- (7) Their germination is favoured by the temperature conditions occurring in nature during the period when they are produced.
- (8) Though less resistant to high temperatures (which do not occur in nature) they are equally resistant to low temperatures.
- (9) The germ tubes penetrate timber with equal facility and rapidity.
- (10) They are equally capable of producing infection in living trees.

The only point in favour of spores appears to be that they retain their vitality for a longer time. Trees diseased by *Ustulina* are usually

infected at or about ground level (though this is not invariable) and this type of infection could be brought about as well by spores as by conidia.

The most significant point in favour of conidial infection is the temperature factor, as shown comparatively in the previous paper (Wilkins, 1938, Fig. 10, p. 74) and in this paper (Fig. 6, p. 78), from which it is evident that, in temperate climates at any rate, the conidia are produced at a time which is favourable for infection whereas spores are produced at a time which is particularly unfavourable.

XII. SUMMARY

1. This paper deals with the germination of the conidia of *Ustilina vulgaris* and the probability of infection of living timber by this means. It is arranged so as to be comparable with the previous paper on spore germination in the same species.

2. The material was collected from beech trees, mostly near Oxford, during a period of six months from April to September in each of three successive seasons. The experimental method is described.

3. Size and shape of conidia are described. The measurements of some 250 conidia indicate that the average size can be expressed as:

$$7.31 \pm 0.003 \times 3.06 \pm 0.0001 \mu.$$

4. Germination in water and various extracts of timber is briefly described.

5. The factors influencing germination were investigated. The physiological condition expressed in terms of "age" was not significant here.

6. The optimum temperature for germination was between 20 and 25° C. The germination range was from 5 to about 30° C. The lower thermal death-point was at an undetermined point below 0° C. and the upper thermal death-point was about 30° C.

7. Germination takes place most satisfactorily in liquids, but will occur to some extent in a saturated atmosphere. In nature, moisture is a most important factor in the production of conidial fructifications and a well-distributed rainfall of at least half an inch per week is essential.

8. Effects of the nutrient aspect of certain media is briefly discussed.

9. The optimum pH was found to be pH 5-6 and the pH range was approximately pH 2-9.

10. Germination in water extracts of seven different timbers showed that germination was better in green than in seasoned timber

extracts, but, with the exception of seasoned oak, germination was generally good.

11. Germ-hyphae were able to penetrate six of the seven timbers, but failed to penetrate oak. Inoculation experiments into young lime and beech trees produced infection but not decay.

12. It is concluded that conidia are the most probable cause of infection of temperate trees by *Ustulina*, and reasons are given for this conclusion.

I am indebted to the Department of Scientific and Industrial Research for a grant enabling me to employ a Research Assistant for this work and I am grateful to the assistant, Miss E. M. Ellis, M.A., B.Sc., for valuable help.

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A CONTRIBUTION TOWARDS A KNOWLEDGE OF INDIAN USTILAGINALES

FRAGMENTS I-XXV

By B. B. MUNDKUR

Imperial Agricultural Research Institute, New Delhi, India

(With 2 Text-figures)

THE first records of Ustilaginales from India were made by M. J. Berkeley (1851, 1854). In those papers, he reported one already known species and four new species, which were included in the collections made by J. D. Hooker in the early part of the last century in north-eastern India. A second report on Indian smuts appeared in 1876 and a third in 1889, both by M. C. Cooke, who reported three new species among the collections made and sent by C. B. Clarke. One of these, however, has now merged into synonymy. Two new species were reported by American workers from Indian material, one by Ellis & Everhart (1890) and another by Ricker (1905); and nine new species were reported by Brefeld (1895), to whom collections were sent by D. D. Cunningham and A. Barclay.

Cunningham (1889) not only sent collections to Brefeld for determination but studied one of them in India. To him belongs therefore the credit for the first careful investigation of a smut in this country. In 1887 he erected the genus *Rhamphopsora* to accommodate a smut found in the leaves of two species of *Nymphaea*; his genus has been merged into *Entyloma* by Setchell (1894).

A more systematic collection of smuts and their identification was undertaken by Dr E. J. Butler at the beginning of the present century, on his appointment as Cryptogamic Botanist to the Government of India. He first endeavoured to identify the collections in India itself with the aid of such literature as was available. The specimens were then numbered and portions sent to H. Sydow for confirmation or correction. As a result thirty new species were named either by Sydow alone or in association with Butler (Sydow & Butler, 1906-12). H. & P. Sydow (1911, 1912) also described five other new species based on material sent from India; and three new species have been named by Indian mycologists (Kulkarni, 1922; Mitra, 1931; and Mundkur, 1938a).

Within recent years smuts have been collected in India by other botanists. Prominent among these are R. R. Stewart and I. D.

Stewart, whose collections were examined by Clinton & Zundel (1938). These contained no new species, but included four until then unrecorded for India.

Of the 109 species of smuts recorded for India in Butler & Bisby (1931), Clinton & Zundel (1938) and Mundkur (1938*b*), fifty-six species were first described from Indian material; the rest were already described from other parts of the world.

In the smuts, as indeed among obligate parasites in general, the tendency among mycologists has been to distinguish species by their ability to parasitize particular hosts. In the identification of a newly collected smut, a search would first be made to see if any species of the genus had already been recorded on that host; if so, the collection would often, after a most cursory examination, be referred to that species. If one had not been so recorded, the fungus was given a new specific name, often without critical and comparative studies of allied species. The correctness of the name applied to a fungus depended on the correctness of the host identification; this was not always correct, and consequently errors crept in.

On the other hand, some mycologists, e.g. G. H. Cunningham of New Zealand (1924), have gone to the other extreme of placing reliance solely on morphological characters, and rejecting other evidence used in differentiating species.

A middle course has been followed in these investigations. Wherever possible the species under study has been compared with the smuts recorded on the same species or nearly allied species of the host plant. Morphological characters, such as spore colour, shape, nature and thickness of the perispore and size, have been given primary importance. Reliance has also been placed on the capacity of the fungus to attack only certain parts of the host. The method of germination of the spores has been used as a diagnostic character of some importance. The capacity of a smut to attack only a certain species of plant has not therefore been made the sole criterion in separating species, though a certain amount of host specialization has been assumed to exist.

This has necessitated the correct identification of the host plants, and the author would like to express his hearty appreciation of the help rendered in identifying the hosts by Dr Kalipada Biswas, Superintendent, and Mr V. Narayanaswami, Curator, Royal Botanic Gardens, Calcutta.

The types and even the cotypes of Indian smuts described in the earlier years by workers outside India are unavailable in India. Later identifications of these smuts have therefore been based on the brief descriptions given in Saccardo. This has frequently led to mis-determinations of the smuts in the *Herbarium Cryptogamae Indiae Orientalis* of the Imperial Agricultural Research Institute at New

Delhi. An endeavour was therefore made to obtain fragments of type specimens for critical comparisons whenever possible. This effort was substantially assisted by Mr S. F. Ashby, Director, Imperial Mycological Institute, Kew, to whom most grateful thanks are here expressed, for the help given in securing the specimens. Full use has been made of the *exsiccatae* of Ustilagineae distributed by Sydow. Fragments of type specimens or authentic *exsiccatae* were also sent on loan by Sir Arthur Hill, Director, Royal Botanic Gardens, Kew, to whom grateful acknowledgements are due.

These efforts have led, after much deliberation and only when fully warranted by the facts, to changes in nomenclature of several Indian smuts.

A uniform set of methods has been followed. Invariably, unless material was scanty, at least 200 spores, mounted in lactophenol, were measured with a Leitz 2 mm. fluorite, oil immersion objective and a $\times 6$ periplanetic micrometer eyepiece. As sunlight is variable from season to season, a 100-watt bulb was used as a source of light, with a very dilute solution of cotton blue as a screen. The conditions of observation have, so far as possible, been standardized.

I. *USTILAGO ANDROPOGONIS-ANNULATI* BREFELD

A smut on *Andropogon annulatus* Forsk. was sent to Brefeld by Cunningham for determination sometime in the early nineties of the last century. Brefeld (1895) compared it with *Schroeteria annulata* Ellis & Everhart, a smut already reported on the same host, concluded that it was a new species and called it *Ustilago Andropogonis-annulati*. The name of the collector, the date of collection and the locality were probably not communicated, for this information is not given on the label of the type specimen deposited by Brefeld in the Herbarium of the Berlin Botanical Museum. Brefeld stated, however, that the specimen had been sent from Dehra Dun which, presumably, is the type locality.

Eleven collections of smuts, made since then at different times and in different localities in India, on the same or nearly allied hosts, have been labelled with this name in the *Herbarium Cryptogamae Indiae Orientalis*. A critical examination of these collections has shown that they contain three species, one of which is new.

The earliest collection of this smut was made by E. J. Butler (No. 462) at Poona in 1902; Sydow & Butler (1906) identified it as *U. Andropogonis-annulati*, presumably after comparison with Brefeld's type specimen. In Brefeld's account of this fungus, more importance was given to the description of the method of germination of the spores, and the conditions necessary to bring this about, than to the morphological characters which are, indeed, very meagrely treated.

The spores furthermore are stated to be $8-9\mu$ in diameter and these incomplete details are repeated in Saccardo (*Sylloge*, xiv, 419).

A good description of *U. Andropogonis-annulati* has been recently given by Zundel (1930) who examined a fragment of the specimen collected by P. A. Pandit (see I (3) below). He transferred it to the genus *Sphacelotheca*, accepting the broader definition of this genus given by Clinton (1904), and with this conclusion I am in agreement. It should be stated that the host plant is now called *Dicanthium annulatum* (Forsk.) Stapf.

Through the courtesy of Dr E. Ulbrich, Kustos and Professor, Berlin-Dahlem Botanical Museum, fragments of Brefeld's type specimens of this and other smuts were kindly obtained for me by Mr S. F. Ashby. A description and the frequencies of the spore measurements are given below.

Sphacelotheca Andropogonis-annulati (Brefeld) Zundel

Ovaricolous. Sori completely destroying the ovaries, later protruding from the glumes by forcing them apart, 3-4 mm. long; covered by a dusky cream false membrane, the cells of which are brownish. Spores subglobose to globose, Kaiser Brown (Ridgway), clearly echinulate under an oil immersion objective, with a diameter range of $7-13\mu$ and mean of 10.1μ .

On *Dicanthium annulatum* (Forsk.) Stapf, and *D. caricosum* (L.) A. Camus, at Dehra Dun, Samalkota, Poona, Nagpur, Andheri and other parts of India including the Punjab. Syn.: *Ustilago Andropogonis-annulati* Bref.

I. (1) Brefeld's type specimen. Morphological characters as above.

Diameter (μ)	8	9	10	11	12	13	
Frequency (n)	1	14	75	104	5	1	=200
Mean= 10.3μ .							

I. (2) Collected by E. J. Butler on *Andropogon annulatus* (= *Dicanthium annulatum*) at Kirkee, Poona, on 9 September 1902. This specimen bears Butler's accession no. 462 and is a part of the specimen examined by Sydow. Morphological characters as above.

Diameter (μ)	8	9	10	11	12	13	
Frequency (n)	1	30	52	102	14	1	=200
Mean= 10.6μ .							

A note left with the specimen by S. N. Mitra states that the spores had a larger diameter than that given by Brefeld, and that this fact had been brought to Sydow's notice.

I. (3) Collected by P. A. Pandit on *A. annulatus* (= *D. annulatum*)

at Nagpur in August 1904. This also, in error, bears Butler's accession no. 462. Morphological characters as above.

Diameter (μ)	8	9	10	11	12	13	
Frequency (n)	3	52	89	44	8	4	= 200
Mean = 10.0 μ .							

I. (4) Collected by R. T. Pearl on *A. caricosus* L. (= *Dicanthium caricosum* (L.) A. Camus) at Nagpur on 24 October 1922. Morphological characters as above.

Diameter (μ)	7	8	9	10	11	12	
Frequency (n)	4	0	79	55	61	1	= 200
Mean = 10.0 μ .							

I. (5) Collected by T. F. Main on *A. annulatus* (= *D. annulatum*) in the Punjab in 1917. Morphological characters as above.

Diameter (μ)	7	8	9	10	11	12	13
Frequency (n)	3	1	45	79	62	6	4 = 200
Mean = 10.2 μ .							

I. (6) Collected by C. A. Barber (no. 920) on *A. annulatus* (= *D. annulatum*) at Samalkota on 3 March 1903. Morphological characters as above.

Diameter (μ)	7	8	9	10	11	12	13
Frequency (n)	1	4	49	64	57	20	5 = 200
Mean = 10.2 μ .							

I. (7) Collected by J. F. Dastur on *A. annulatus* (= *D. annulatum*) at Andheri, Bombay. Morphological characters as above.

Diameter (μ)	7	8	9	10	11	12	
Frequency (n)	9	26	43	59	54	9	= 200
Mean = 9.9 μ .							

I. (8) Collected by P. A. Pandit on "*Andropogon* sp." at Nagpur on 26 July 1907. As the conception of the genus *Andropogon* has been considerably changed in recent years, it is doubtful if the host is in fact an undetermined species of *Andropogon*. Morphological characters as above.

Diameter (μ)	8	9	10	11	12	13	
Frequency (n)	5	30	62	79	20	4	= 200
Mean = 10.4 μ .							

II. *SPHACELOTHECA ANNULATA* (ELLIS & EVERHART) MUNDKUR

Three collections of smuts on *Andropogon annulatus* (= *Dicanthium annulatum* (Forsk.) Stapf) and labelled *Ustilago Andropogonis-annulati* Brefeld (see previous note) in the *Herbarium Cryptogamae Indiae Orientalis* were found, on critical examination, to have larger spores

and to differ in certain essential characters from the type specimen of *Sphacelotheca Andropogonis-annulati*. They agree, however, with *Ustilago Duthiei* Ricker.

Ustilago Duthiei was collected by J. F. Duthie on a grass then designated *Andropogon Bladhii* Retz. (= *D. annulatum*) at Dehra Dun on 22 October 1888. It is not known how the specimen found its way to the United States of America, where Ricker (1905) described it. Ricker fortunately stated that the specimen bore Duthie's field no. 7699, and with this clue the type was traced to the University of Wisconsin and the cotypes to Dehra Dun and to Kew. Fragments of the former were kindly sent by Dr E. M. Gilbert of that University and of the cotypes by N. L. Bor, Forest Botanist, Dehra Dun and by Sir Arthur Hill. Duthie's plant bearing his field no. 7699 is not a grass. Ricker's type specimen in the Herbarium of the University of Wisconsin, however, bears the no. 7679, and so do the cotypes at Kew and at Dehra Dun, where on the sheet of the specimen Duthie has written "ergotized".

The cotypes received from Dehra Dun and Kew agreed very well with Ricker's type, and there is now no doubt that among the specimens in the *Herbarium Cryptogamae Indiae Orientalis* three labelled *Ustilago Andropogonis-annulati* are really *U. Duthiei*. Zundel (1930) transferred *U. Duthiei* to the genus *Sphacelotheca*, a change with which I am in agreement.

At the time this smut was under investigation by me, an *exsiccata* of *Schroeteria annulata* was received from F. J. Seaver of the New York Botanic Garden through the courtesy of S. F. Ashby. This smut was described and named by Ellis and Everhart (1890) from the ovaries of *Andropogon annulatus*. The collector is stated to be S. M. Tracy, an American mycologist, but neither the exact locality in India nor the date of collection is given on the type specimen in the New York Botanic Garden. Ellis and Everhart stated that the spores were in twos or occasionally threes, flattened on the line of contact, hyaline, 12–15 μ in diameter at first, becoming brown and separating into two distinct spores, 7–10 μ in diameter; they placed the smut in the genus *Schroeteria*. It is not clear if Brefeld (1895) had actually seen this smut when he compared his *Ustilago Andropogonis-annulati* with it.

An examination of the specimen has not shown any hyaline spores. The spores, which are brown, do not occur in twos or occasional threes, but they are of the usual *Sphacelotheca* type. Ellis and Everhart either mistook for mature spores the sterile cells of the membrane, which are hyaline, and sometimes occur in chains or groups, or mistook the host tissue or immature spores for them. When the spores were compared with the spores of *Schroeteria Delaestrina* (Tul.) Winter (Sydow, Ustilagineae, no. 336), the species on which the

II. (3) Collected by Inayat Khan on *A. annulatus* (= *D. annulatum*) at Dehra Dun on 21 June 1904. Morphological characters as above.

Diameter (μ)	9	10	11	12	13	14	15	
Frequency (n)	13	28	102	33	18	4	2	= 200

Mean = 11.3 μ .

II. (4) Collected by S. Sundararaman on *Dicanthium annulatum* at Coimbatore on 29 May 1935. Morphological characters as above.

Diameter (μ)	9	10	11	12	13	14	15	
Frequency (n)	18	38	96	33	14	0	1	= 200

Mean = 11.0 μ .

II. (5) Collected by H. M. Chibber (no. 228) on *A. annulatus* (= *D. annulatum*) at Bassein on 22 November 1908. Morphological characters as above.

Diameter (μ)	9	10	11	12	13	14	15	
Frequency (n)	22	46	82	29	15	3	3	= 200

Mean = 11.0 μ .

III. A NEW SMUT ON *DICANTHIUM ANNULATUM*

A smut on *Andropogon annulatus* (= *Dicanthium annulatum*) was collected by E. J. Butler on 30 August 1905 at Chatrapur, Ganjam District, and the specimens in the *Herbarium Cryptogamae Indiae Orientalis* were labelled *Ustilago Andropogonis-annulati*. On comparing this smut with the type specimens of *Sphacelotheca Andropogonis-annulati* and *S. annulata* (see Notes I and II), it at once became manifest that the identification was incorrect. As it does not agree with any of the smuts known on this or nearly allied hosts, it is no doubt a new species. It belongs to the genus *Sphacelotheca* and is named *S. Sahayai* after Mr C. B. Sahaya, the author's colleague who has rendered considerable help in these investigations.

Sphacelotheca Sahayai Mundkur spec.nov.

Soris in ovariis evolutis, ovoideis, ad 2 vel 3 mm. longis, primo inter glumas compressas occultis, deinde inter eas patentes manifestis, massam sporarum semi-agglutinatam, nigrobrunneam membrana evanescenti tegentibus, postremo columellam prominentem, nudatam exhibentibus. Cellulis sterilibus, hyalinis, irregularibus, nonnihil angulatis et sporis magnitudine praestantibus. Sporis constanter globosis, Vandyke Brown (Ridgway), epispora crassissima propter aculeos magnos et extantes quasi serrata praeditis, 9-15 (in medio 12.2) μ diam.

Hab. In ovariis *Dicanthii annulati* (Forsk.) Stapf, Chatrapur, Ganjam District, Indiae or. (E. J. Butler; Aug. 1905.)

Sori ovaricolous, ellipsoidal, about 2–3 mm. long, at first hidden by the glumes but later becoming manifest as the glumes spread; covered by an evanescent membrane which encloses the semi-agglutinated blackish brown spore mass; as the membrane and the spores wear away, displaying a prominent columella. Sterile cells hyaline, irregular, slightly angled, and larger than the spores. Spores Vandyke Brown (Ridgway), regularly globose, with prominent and large echinulations that give a serrated appearance to the edges; epispore very thick; diameter ranges from 9 to 15 with a mean of 12.2 μ .

On *Dicanthium annulatum* (Forsk.) Stapf, at Chatrapur, Ganjam District. Specimens of original material deposited in the *Herbarium Cryptogamae Indiae Orientalis* at New Delhi and at the Imperial Mycological Institute, Kew.

The spores of this smut differ from those of *Sphacelotheca Andropogonis-annulati* in having a larger diameter, a thicker epispore and more prominent echinulations. They differ from the smooth spores of *S. annulata* in having a larger diameter and a thicker, echinulate epispore. They are deeper brown than the spores of the other two species.

III. (1) Collected by E. J. Butler on *Dicanthium annulatum* at Chatrapur, Ganjam District, on 30 August 1904. *Type*.

Diameter (μ)	9	10	11	12	13	14	15	
Frequency (<i>n</i>)	6	13	103	39	21	7	11	=200

Mean = 12.2 μ .

IV. *USTILAGO SUPERFLUA* SYD.

This smut was collected by F. J. F. Shaw on *Andropogon foveolatus* Del. at Samalkota on 21 November 1910. The specimen in the *Herbarium Cryptogamae Indiae Orientalis* bears E. J. Butler's accession no. 1431, and is a part of the specimen submitted to Sydow (see Sydow & Butler, 1906) for determination. The accepted name of the host plant is *Eremopogon foveolatus* (Del.) Stapf.

Sphacelotheca superflua (SYD.) ZUNDEL

Inflorescence entirely destroyed. Sori at first hidden by the leaf sheath but later protruding; deep dark brown, 5–14 mm. long, covered by a false membrane which gradually disintegrates; provided with a prominent columella, seen on teasing the sorus. Cells of the membrane hyaline or slightly tinted, smooth and sterile. Spores Kaiser Brown (Ridgway) and slightly opaque, subglobose but often irregular in outline and frequently angled; epispore thin, covered with prominent echinulations that give the edge a serrated appearance.

The smut was transferred to *Sphacelotheca* by Zundel (1930), and this appears to be justifiable only so far as our present knowledge of the smut goes. The appearance of the sorus, the size of the spores and the prominent echinulations which can almost be termed spines, and the fact that many of the spores are subopaque, lead to the suspicion that it may belong to *Tilletia*. Species belonging to that genus, however, usually have a thick epispore, while in the present fungus the epispore is rather thin. The method of germination of the spores is of course the chief distinguishing generic character and until that has been observed, the smut can only be tentatively placed in the genus *Sphacelotheca*. Syn.: *Ustilago superflua* Syd.

IV. (1) Collected by F. J. F. Shaw on *Andropogon foveolatus* (= *Eremopogon foveolatus*) at Samalkota on 21 November 1910. Cotype.

Diameter (μ)	10	11	12	13	14	15	16	
Frequency (n)	1	6	29	106	38	16	4	= 200

Mean = 13.2μ .

V. *USTILAGO NARDI* SYD.

A smut on *Andropogon Nardus* Hooker f. (non L.) was collected by E. J. Butler at Vayitri, Wyanaad Hills, Malabar, on 2 November 1904, and described as a new species, *Ustilago Nardi* by Sydow (in Sydow & Butler, 1906). A duplicate of Butler's no. 470, on which Sydow founded the species, is in the *Herbarium Cryptogamae Indiae Orientalis* at New Delhi. A second collection was made by W. McRae at Panora, Wyanaad Hills, on 17 November 1909.

Petch (1912) suggested that this species might be *Ustilago spermoidea* of Berkeley & Broome (1875), recorded on *Cymbopogon Martini*, which, as understood by Thwaites, is a synonym of *Andropogon Nardus*.

Specimens of the host plant collected by Butler (no. 470) were submitted to the Royal Botanic Gardens, Calcutta, for redetermination, and it was stated, in reply, that the host plant was *Cymbopogon confertiflorus* (Steud.) Stapf (= *Andropogon Nardus* var. *confertiflorus*), as was also the specimen collected by McRae.

It will be noted that neither of the Indian specimens is on *Cymbopogon Martini*, and Petch's suggestion (1912) that *Ustilago Nardi* may be *U. spermoidea* was tested by comparison of the two smuts. A cotype of the latter species (Thwaites no. 589) was kindly sent by W. R. C. Paul, mycologist, Peradeniya, Ceylon, and critical examination has shown that in *U. spermoidea* the spores are larger, deeper brown and have thicker episporae. In my judgement, these two smuts are not the same species. Zundel (1938) transferred *U. Nardi* to the genus *Sphacelotheca* on valid grounds. Berkeley and Broome's smut also belongs to *Sphacelotheca* on account of its papyrus-like false membrane and a columella which is, however, rather indistinct.

Sphacelotheca Nardi (Syd.) Zundel

Sori ovaricolous, 2-3 mm. long, linear, covered with a delicate false membrane which later disintegrates revealing a brown spore mass and a well-developed columella, nearly concealed by the leaf-sheath. Sterile cells in groups or chains, rectangular, brownish and $4.5-7\mu$ in diameter. Spores globose to subglobose, irregular, Carob Brown (Ridgway), smooth, ranging in diameter from 6 to 10μ with a mean of 7.7μ ; epispore not thick. Syn.: *Ustilago Nardi* Syd.

V. (1) Collected by E. J. Butler on *Andropogon Nardus* (= *Cymbopogon confertiflorus*) at Vayitri, Wyanaad Hills, Malabar, on 2 November 1904. Butler's no. 470, which is a part of the type specimen, was examined. Morphological characters as above.

Diameter (μ)	6	7	8	9	10	
Frequency (n)	16	120	46	17	1	= 200

Mean = 7.6μ .

V. (2) Collected by W. McRae on *Andropogon Nardus* (= *Cymbopogon confertiflorus*) at Panora, Wyanaad Hills, on 17 November 1909. Morphological characters as above.

Diameter (μ)	6	7	8	9	10	
Frequency (n)	7	93	48	48	5	= 201

Mean = 7.7μ .

Sphacelotheca spermoidea (B. & Br.) Mundkur comb.nov.

Sori ovaricolous, 4.5 mm. long, at first hidden by the glumes but later somewhat protruding; covered by a false membrane of papyrus-like consistency, which may later flake away. Columella rather indistinct. Spores globose to subglobose or slightly irregular with a thick but smooth epispore, Kaiser Brown (Ridgway); diameter ranging from 7 to 12μ with a mean of 9.8μ . Syn.: *Ustilago spermoidea* B. & Br.

V. (3) Collected by Thwaites (no. 589) on *Cymbopogon Martini* in Ceylon. *Cotype*.

Diameter (μ)	7	8	9	10	11	12	
Frequency (n)	1	3	56	86	52	2	= 200

Mean = 9.8μ .

VI. *USTILAGO SCHOENANTHI* SYD. & BUTLER

This smut was found by C. A. Barber (no. 3347) on "*Andropogon Schoenanthus* L." at Alanacolum, Tinnevely District, on 1 July 1901 and sent to E. J. Butler for determination. The specimen in the *Herbarium Cryptogamae Indiae Orientalis* bears Butler's accession no. 449 and is a part of that on which Sydow and Butler (1906) founded

the species, *Ustilago Schoenanthi*. An earlier specimen of "*A. Schoenanthus*" collected by Barber (no. 2677) at Palamcottā, Tinnevely District, on 11 May 1901, also bears, in error, the same accession no. 449. A third specimen of the same host, collected at Bargur, Krishnagiri, Salem District, on 31 October 1924, is also labelled *Ustilago Schoenanthi*. Zundel (1930) has transferred the smut to the genus *Sphacelotheca* on grounds which I consider as valid.

A redetermination of the grasses by the Curator, Royal Botanic Gardens, Calcutta, showed that the specimens from Alanacolam and from Bargur were *Cymbopogon flexuosus* (Steud.) Watson, and that the specimen from Palamcottā was *C. coloratus* Stapf. Examination of the smut affecting the latter specimen showed that that smut was not *Ustilago Schoenanthi* but an undescribed species.

A critical examination of part of the type specimen (no. 449) and the collection from Bargur has shown good agreement with the description given by Sydow & Butler (1906), but considerable disagreement with the description given by Zundel (1930).

Sphacelotheca Schoenanthi (Syd. & Butler) Zundel

Sori ovaricolous and completely destroying the ovaries. About 10-15 mm. long with a false membrane which finally disintegrates, revealing a dark brown non-granular spore-mass. Columella non-flattened, simple or multiple, hidden at first by the sheath; sterile tissue breaking up into large, hyaline, subglobose cells. Spores regular, globose to subglobose, Carob Brown (Ridgway) with a very thick epispore which is smooth (not echinulate as stated by Zundel); diameter ranges from 5 to 9 μ with a mean of 7.0 μ . Syn.: *Ustilago Schoenanthi* Syd. & Butler.

On *Cymbopogon flexuosus* (Steud.) Watson (= *Andropogon Nardus* Hooker f. var. *flexuosus* Steud. but at first incorrectly identified as *A. Schoenanthus* L.) at Alanacolam, Tinnevely District, and Bargur, Krishnagiri, Salem District.

VI. (1) Collected by C. A. Barber (no. 3347) on "*Andropogon Schoenanthus*" (real name = *Cymbopogon flexuosus*) at Alanacolam, Tinnevely District. Butler's no. 449. Type. Morphological characters as above.

Diameter (μ)	5	6	7	8	9	
Frequency (n)	3	54	118	15	10	= 200
Mean = 7.0 μ .						

VI. (2) Collected on *A. Schoenanthus* (real name = *Cymbopogon flexuosus*) at Bargur, Krishnagiri, Salem District, on 31 October 1924.

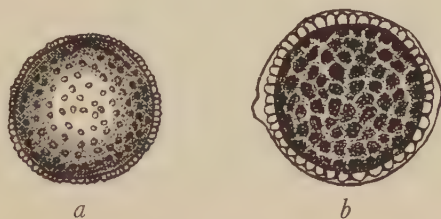
Diameter (μ)	5	6	7	8	
Frequency (n)	12	118	69	1	= 200
Mean = 6.1 μ .					

VIII. *NEOVossIA* *BARCLAYANA* BREFELD

A smut collected on *Pennisetum triflorum* Nees (= *P. orientale* Rich.) at Simla on 30 August 1889 by A. Barclay was sent for determination to Brefeld (1895) who found that it was an undescribed species of *Neovossia* and named it *N. Barclayana*. An examination of a fragment of Brefeld's type specimen has been made and a fresh description is given below.

Neovossia Barclayana Brefeld

Ovaricolous; sori bullate, covered by a firm membrane which consists of large, hyaline, smooth, sterile cells with very thick walls and encloses a pulverulent, black, spore-mass. Spores ellipsoidal to



Text-fig. 1. a. Chlamydospore of *Neovossia Barclayana* $\times 1120$. b. Chlamydospore of *Tilletia Pennisetina* $\times 960$.

subglobose, subopaque (but never completely so), Vandyke Brown (Ridgway) and without an appendage; with extremely minute warts which can be seen with great difficulty at the edges of the epispor. Diameter ranges from 15.5 to 22.0μ with a mean of 18.3μ .

VIII. (1) Collected by A. Barclay on *Pennisetum triflorum* (= *P. orientale* Rich.) at Simla on 30 August 1889. Morphological characters as above. *Type*.

Diameter (μ)	15.5	16.8	18.1	19.4	20.7	22.0	
Frequency (n)	9	22	34	18	12	5	= 100
Mean = 18.3μ .							

In 1899 Saccardo and Sydow (*Sylloge Fungorum*, xiv, 422) transferred this species to *Tilletia*, as Winter (1881) did not accept the genus *Neovossia*. But that genus is considered valid by Masee (1899), Magnus (1900), Schellenberg (1911) and Dietel (1928), and Saccardo and Sydow (*Sylloge Fungorum*, xvi, 375) have restored the species to the genus *Neovossia*.

As late as 1935 Yen used the name *Tilletia Barclayana* Sacc. & Syd., but his fungus, on *Pennisetum alopecuroides* (L.) Spreng., really agrees with *Tilletia Pennisetina* Sydow rather than with *Neovossia Barclayana*, as will be apparent from the perusal of the descriptions by Yen (1935) and by Sydow (1929).

Sydow (1929) states that Brefeld's fungus has somewhat brighter spores with a smaller diameter, and a thinner epispore than those of his *Tilletia Pennisetina*; further, that the sterile cells of *Neovossia Barclayana* are very large, smooth, and extremely thick-walled. These features were all present in the fragment examined by me.

A search for more material of *Neovossia Barclayana* on *Pennisetum orientale* in the vicinity of Simla is very desirable.

IX. *TILLETIA ELEUSINES* SYD.

The earliest collection of this smut was made by L. S. Subramaniam on *Eleusine aegyptiaca* Desf. at Pusa (Bihar) on 12 October 1914. He found that the spores ranged in diameter from 21 to 29 μ , and that the fungus was an undescribed species of *Tilletia*, but he failed to name it. The next collection was made on 18 November 1914 at Coimbatore (Madras) by the Farm Superintendent of that place, and a third was made at Sialkot (Punjab) on 8 September 1917 by G. S. Cheema.

Sydow (1935) described it as a new species, *Tilletia Eleusines*, from a specimen collected on the same host at Lahore in 1932 by H. Chaudhuri. It has a wide geographic range, as it is recorded from such different stations as Bihar, Madras and the Punjab.

The host plant does not, according to Blatter & McCann (1935), belong to *Eleusine*, and its accepted name is *Dactyloctenium aegyptium* Richt. It has been placed at different times in such genera as *Aegilops*, *Cenchrus*, *Chloris*, *Cynosurus*, *Eleusine* and *Rhabdochloa*. A search in Saccardo and other literature showed that no species of *Tilletia* has so far been reported on any of these with the exception of *Tilletia caries* (DC.) Tul. on *Aegilops*. The smut on *Dactyloctenium aegyptium* does not agree, however, with that species.

A fragment of the type specimen was kindly sent to me by Sydow and with this have been compared the earlier collections, all of which undoubtedly belong to this species.

Tilletia Eleusines Syd.

Sori formed in the ovaries which are completely destroyed, almost globose, oval, or oblong, olive-green, 2-3 mm. long; distributed unevenly in the spike, remaining single or several together. Spore-mass blackish. Spores globose, dark brown, 17-28 μ in diameter, with a mean of 21.0 μ . Epispore 2.5-3.5 μ thick with raised ridges, forming four- to six-angled reticulations, 3-5 μ broad; a smooth hyaline membrane can be made out in some of the spores. Sterile cells irregularly globose, hyaline, smooth, 8-14 μ in size with a 1.5-2 μ thick wall.

On *Dactyloctenium aegyptium* Richt. (= *Eleusine aegyptiaca* Desf.), at Pusa, Coimbatore, Sialkot and Lahore.

IX. (1) Collected by H. Chaudhuri on *Eleusine aegyptiaca* (= *D. aegyptium*) at Lahore in 1932. Type.

Diameter (μ)	17	18	19	20	21	22	23	24	25	26	27	28
Frequency (n)	4	17	20	35	38	42	21	10	7	3	2	1 = 200

Mean = 21.1 μ .

IX. (2) Collected by L. S. Subramaniam on same host at Pusa on 12 October 1914. Morphological characters as above.

Diameter (μ)	17	18	19	20	21	22	23	24
Frequency (n)	3	3	34	36	72	27	22	3 = 200

Mean = 20.6 μ .

IX. (3) Collected on same host at Coimbatore on 11 November 1914. Morphological characters as above.

Diameter [†] (μ)	18	19	20	21	22	23	24	25	26
Frequency (n)	1	17	13	91	35	30	7	5	1 = 200

Mean = 21.2 μ .

IX. (4) Collected by G. S. Cheema on same host at Sialkot on 8 September 1917. Morphological characters same as above.

Diameter (μ)	18	19	20	21	22	23	24	25	26	27
Frequency (n)	1	7	27	102	31	14	9	5	3	1 = 200

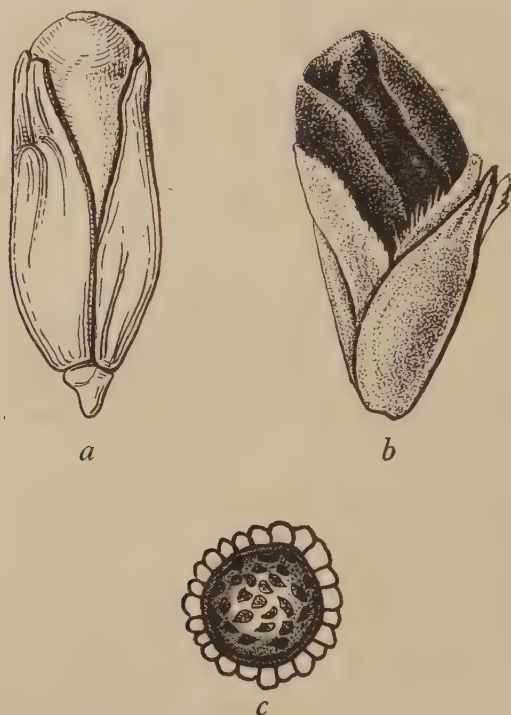
Mean = 21.4 μ .

X. A NEW SPECIES OF *TILLETIA* ON BAJRA, *PENNISETUM TYPHOIDES* STAPP (PEARL MILLET)

While conducting experiments on the Bajra smut, *Tolyposporium Penicilliariae* Bref., to determine the mode of its transmission, S. L. Ajrekar, Professor of Botany, Gujerat College, Ahmedabad, found a single grain of this cereal attacked by a species of *Tilletia*. The experiments were conducted in pots containing soil infested by *Tolyposporium* and were sown with seeds that had been obtained from

the College of Agriculture, Poona. These seeds were harvested from a crop in which no smut was present.

The panicles, as they emerged, were carefully examined by Ajrekar to detect the appearance of *Tolyposporium*. In one panicle he found a single grain that seemed to be attacked by some smut, but the chlamydospores proved to be those of a *Tilletia*. Examination of other panicles to collect more sori of this *Tilletia* proved futile and



Text-fig. 2. *a.* Healthy grain of Bajra, *Pennisetum typhoides* $\times 6$. *b.* Smutted grain of *Pennisetum typhoides* $\times 6$. *c.* Chlamydospore of *Tilletia Ajrekari* $\times 1120$.

careful search of Bajra fields at Ahmedabad has so far been fruitless. The smut was kindly placed at my disposal for critical study.

One species of *Tilletia*, *T. Pennisetina* Syd., on *Pennisetum alopecuroides* in China and another, of the allied genus *Neovossia*, *N. Barclayana* Bref. on *Pennisetum orientale* in India have so far been reported on the genus *Pennisetum* (Ciferri, 1931). The specimen on Bajra has been compared with the types of these two species and also with other species of *Tilletia* occurring in India. Descriptions of species of *Tilletia* given in Massee (1899) and Saccardo (85 species) have been examined to see if the smut on Bajra agreed with any

previously described species. There is little doubt that it has not been described and the name *Tilletia Ajrekari*, in honour of the collector, is proposed for it.

***Tilletia Ajrekari* Mundkur spec.nov.**

Soro ovarium omnino destruenti, e glumis extanti, ovoideo, ad 6 mm., longo, demum contracto et corrugato, membrana atra, crassa, tenaci, apice dehiscendi praedito. Cellulis sterilibus hyalinis, levibus, pariete modice crassa praeditis, 14–17 μ diam. Sporis pulverulentis, plerumque globosis, raro diversis, "Diamine Brown" (Ridgway), translucetibus, aculeis, vel vero verrucis, complanatis, subrectangularibus, valde irregulariter dispositis ornatis, 13.8–21 (in medio 17.7) μ diam.

Hab. In ovario Penniseti typhoidis Stapf, in agellis experimento dedicatis, Ahmedabad, Indiae or. (S. L. Ajrekar; Sept. 1933. Typus est sorus unicus.)

Ovaricolous. Sorus completely destroying the ovary and protruding prominently from the glumes, ellipsoidal, about 6 mm. long, slightly folded upon itself and shrunk; clothed with a black, thick and firm membrane enclosing the spores, which escape from a slit formed at the apex. Spores pulverulent, mostly globose but occasionally aberrant; deep brown but by no means opaque, with an epispore 2–3 μ thick on which flattened, roughly rectangular, very irregularly placed, blunt spines or verrucae occur; diameter 13.8–21 μ with a mean of 17.7 μ . Sterile cells hyaline, smooth, with a not very thick wall, 14–17 μ in diameter. Germination unknown.

On *Pennisetum typhoides* Stapf (Bajra, pearl millet) in experimental pots at Ahmedabad. Type specimen (a single sorus) deposited in the Imperial Agricultural Research Institute, New Delhi, India.

X. (1) Collected by S. L. Ajrekar on *Pennisetum typhoides* at Ahmedabad on 2 September 1933. *Type.*

Diameter (μ)	14	15	16	17	18	19	20	21	
Frequency (n)	4	7	24	70	33	27	22	13	=200

Mean = 17.7 μ .

A fragment of the type specimen of *Tilletia Pennisetina* was kindly sent by Sydow. Macroscopically it resembled the sorus of *T. Ajrekari*, but under the microscope the spores were found to be more deeply dark brown to completely opaque, much larger, and with less blunt verrucae, arranged regularly on the epispore. These verrucae are smaller than those of *T. Ajrekari* but are larger than those of *Neovossia Barclayana*. The species of *Tilletia* which Yen (1935) has illustrated and determined as *T. Barclayana* is, in my opinion, *T. Pennisetina*. Spore measurements of *T. Pennisetina* are given below.

X. (2) Collected in ovaries of *Pennisetum alopecuroides* (L.) Spreng., in the province of Kiangsi, China. *Type*.

Diameter (μ)	16	18	20	22	24	26	28	
Frequency (n)	11	32	66	47	36	7	1	=200

Mean = 21.3 μ .

It should be noted that *Neovossia Barclayana* has chlamydospores which are slightly larger in diameter, spines which are smaller and very regularly arranged, and larger sterile cells which have a thicker wall than those of *Tilletia Ajrekari*.

The sudden appearance of a single sorus of a hitherto unknown species of *Tilletia* in this millet is a biological problem which needs further investigation. Its sorus resembles somewhat those of *Tolyposporium Penicilliariae* for which it may be mistaken, and an examination of each and every smut sorus in panicles of Bajra in the fields in Gujerat is therefore desirable. The species seems very rare.

XI. *USTILAGO AMADELPHA* SYD. & BUTLER

A culmicolous smut, collected at Awapur, Muzaffarpur District, on a supposed species of *Andropogon*, on 15 April 1911, was described by Sydow & Butler (1912) as a new species, *Ustilago amadelpha*. Butler's specimen (no. 1425), which is a portion of the type collection, is available in the *Herbarium Cryptogamae Indiae Orientalis*. So far as is known, the smut has only been once collected. It has recently been redescribed by Zundel (1930).

Ustilago amadelpha Syd. & Butler

Culmicolous, destroying the entire inflorescence which is turned into a flagellum; at first hidden by the sheath, curled, or slightly twisted. Sori 2–12 cm. long, covered by a silvery membrane, and strikingly resembling the whip of the sugar-cane smut. Spores globose to subglobose, black in mass, prominently echinulate, so that the edge of the epispore appears slightly serrate; Chestnut (Ridgway), with a thick epispore; diameter of spores ranging from 5 to 9.5 μ with a mean of 7.9 μ .

On *Andropogon* (?) species at Awapur, Muzaffarpur District.

XI. (1) Collected at Awapur, Muzaffarpur District, on *Andropogon* (?) sp. on 15 April 1911. *Type* (no. 1425).

Diameter (μ)	5	6	7	8	9-9.5	
Frequency (n)	2	5	98	58	37	=200

Mean = 7.9 μ .

A note accompanying the specimen states that the grass had no flowers and might be a species of *Saccharum*. An endeavour was made to get the specimen re-identified. The Curator of the Royal Botanic

Gardens, Calcutta, wrote that the specimen was so imperfect that it "may be anything". The Forest Botanist at Dehra Dun was of a similar opinion. The host genus of this smut must be considered, therefore, as undetermined.

A culmicolous smut, also on an undetermined grass from Java, was considered by Ciferri (1933) to be a new variety of this species. Ciferri hazarded the opinion that the host might be a species of *Andropogon*.

XII. *USTILAGO ELEUSINIS* KULKARNI

A smut on nachni, *Eleusine coracana* Gaertn. f., was collected by G. S. Kulkarni at Malkapur in October 1918. He studied the smut (Kulkarni, 1922), concluded that it was a new species and called it *Ustilago Eleusinis*. An *exsiccata* of this smut, collected in its type locality two years later and identified by Kulkarni, was kindly sent to me by B. N. Uppal, Plant Pathologist, Poona.

The sorus is covered by a membrane but there is no columella. The spores have a rough and minutely pitted episporium, and these pits give the margin an uneven appearance, which Kulkarni evidently mistook for spines or echinulations.

Ustilago Eleusinis Kulkarni

Ovaricolous and scattered in the spike, entirely destroying the ovaries with the exception of the ovary wall. Sori 3–15 mm. long, greenish at first but deep brown at maturity, covered by the ovary wall and without any columella. Spores pulverulent, globose to subglobose, Auburn (Ridgway); epispore densely pitted, with a rather uneven margin diameter ranging from 7 to 11 μ with a mean of 9.6 μ .

On *Eleusine coracana* Gaertn. (in cultivation) at Malkapur and other parts of the Bombay Presidency.

XII. (1) Collected by G. S. Kulkarni on *Eleusine coracana* at Malkapur, Bombay Presidency, on 26 October 1920. Authentic specimen.

Diameter (μ)	7	8	9	10	11	
Frequency (n)	9	27	60	75	29	= 200
	Mean = 9.6 μ .					

XII. (2) A smut on the same host was collected by J. McDonald at Nairobi, Kenya, on 21 October 1936, and sent for determination. It agrees very well with Kulkarni's fungus excepting that its spores have a wider range and a larger mean.

Diameter (μ)	8	9	10	11	12	13	14	
Frequency (n)	3	4 ¹	65	65	18	4	4	= 200
	Mean = 10.4 μ .							

Sydow (1929) described a culmicolous smut on *Eleusine indica* Gaertn., from China, to which he gave the name *Ustilago Eleusines*. The spores of this smut are echinulate and of smaller diameter than those of the foregoing. It is presumably a different species, but Sydow's specific epithet is not valid as it had already been used by Kulkarni.

XIII. *USTILAGO SPARSA* UNDERWOOD

L. S. Subramaniam collected a smut on *Eleusine aegyptiaca* Desf. at Pusa on 4 November 1914, and a second collection on the same host was made four years later. A specimen of this grass affected by a smut was also received recently from the Herbarium, Department of Agriculture, Victoria, Australia, for identification. It was collected by G. F. Hill at Darwin on 2 April 1916. The smuts in all the three collections agree among themselves in all major respects.

Eleusine aegyptiaca is a synonym of *Dactyloctenium aegyptium* Richt., and three species of *Ustilago* have so far been reported on *Dactyloctenium*. A smut on *D. aegyptium* in East Africa was described by P. Hennings (1895) as *Ustilago Dactyloctenii*. This is a culmicolous smut, entirely destroying the inflorescence and with spores of diameter $7-14\mu$ (see Zundel, 1938). The smut found at Pusa and at Darwin is, on the contrary, ovaricolous with spores ranging in diameter from 6 to 10μ and is therefore not *U. Dactyloctenii*. Another smut, *U. Dactylocteniphila*, on *Dactyloctenium mucronatum* from Brazil, also described by P. Hennings, is ovaricolous, but its spores are $9-14 \times 8-13\mu$ in diameter and possess smooth episporous. The Pusa and the Darwin specimens agree, however, with the third smut *Ustilago sparsa* Underwood (1897), on *Dactyloctenium aegyptium*, in all respects and are undoubtedly that species.

Ustilago sparsa Underwood

Ovaricolous, infesting ovaries scattered here and there in the spike and entirely destroying them; sori 2-3 mm. long, olivaceous but later disclosing a dusty, brown-black spore-mass. Spores Morocco Red (Ridgway), globose to oval, echinulate (more distinct at the margin than on the surface), $6-10\mu$ in diameter with a mean of 7.8μ .

On *Dactyloctenium aegyptium* Richt. (= *Eleusine aegyptiaca* Desf.) at Auburn, Alabama (U.S.A.), Pusa (India), and Darwin (Australia).

XIII. (1) Collected by L. S. Subramaniam on *Dactyloctenium aegyptium* at Pusa on 4 November 1914.

Diameter (μ)	6	7	8	9	10	
Frequency (n)	8	101	65	25	1	= 200
		Mean = 7.8μ .				

XIII. (2) Collected by L. S. Subramaniam at Pusa on the same host on 14 November 1918. Morphological characters as above.

Diameter (μ)	6	7	8	9	
Frequency (n)	16	95	57	32	= 200
Mean = 7.6 μ .					

The specimen collected on 2 April 1916 by G. F. Hill at Darwin and sent by the Herbarium, Victoria Department of Agriculture, gave the following measurements:

Diameter (μ)	6	7	8	9	10	
Frequency (n)	10	72	78	33	7	= 200
Mean = 7.8 μ .						

An *exsiccata* of *Ustilago sparsa* from Seymour and Earle's *Economic Fungi*, Clinton Ust. Supp. C 86, received from the Herb. Hort. Bot. Reg. Kew., gave the following measurements:

Diameter (μ)	6	7	8	9	10	
Frequency (n)	4	94	59	40	3	= 200
Mean = 7.8 μ .						

A specimen of Ravenel's Fungi Amer. no. 790 was also examined and found to be the same as *Ustilago sparsa*.

XIV. *USTILAGO COICIS* BREFELD

Smutted plants of *Coix Lachryma-Jobi* Linn. collected in the vicinity of Simla in February 1891 were sent by Barclay to Brefeld for determination. Brefeld (1895) found that the ovaries were not only entirely destroyed but that all in the raceme were smutted; compared with healthy ones, however, they were not much swollen. He called the fungus *Ustilago Coicis*. The smut has been reported in Java. The two later collections from India, cited by Butler & Bisby (1931) are different smuts. A fragment of the type specimen has been examined and Brefeld's (1895) description, which is rather brief, is emended as follows.

Ustilago Coicis Brefeld

Ovaricolous, completely destroying the ovaries, all of which in a raceme are destroyed. Sori 9-13 mm. long and 5-9 mm. broad, brown-black and containing a pulverulent spore-mass. Spores held together by the hard floral glumes, Liver Brown (Ridgway) subglobose to ellipsoidal with minute but clear echinulations which give the margin a serrate appearance; rather prominent, circular pits also present. Diameter ranges from 7 to 13 μ with a mean of 9.2 μ .

On *Coix Lachryma-Jobi* Linn. near Simla.

XIV. (1) Collected by A. Barclay on *Coix Lachryma-Jobi* near Simla in February 1891. *Type*. Morphological characters as above.

Diameter (μ)	7	8	9	10	11	12	13	
Frequency (n)	7	17	108	55	6	6	1	= 200

Mean = 9.2μ .

XV. *USTILAGO BENGALENSIS* SYD. & BUTLER

Cymbopogon pendulus Stapf, affected by a smut, was collected by I. H. Burkill at Banarhat, Doars, Bengal, on 8 September 1908. The grass was so badly affected by the smut that Burkill seems to have had difficulty in getting healthy specimens for study. A specimen of the smutted grass was sent to the *Herbarium Cryptogamae Indiae Orientalis* for the determination of the fungus, which Sydow & Butler (1912) found to be a new species and called *Ustilago bengalensis*.

The smut clearly belongs to the genus *Sphacelotheca*, as the sori possess an evanescent false membrane consisting of smooth, hyaline sterile cells and a prominent, dark brown columella nearly 1 cm. long.

Sphacelotheca bengalensis (Syd. & Butler) Mundkur comb.nov.

Ovaricolous, sori entirely destroying the ovaries, linear, 5–10 mm. long, with a long, simple columella, and covered by an evanescent false membrane which flakes away into sterile, hyaline cells. Spores pulverulent, dark brown in mass, globose to subglobose, Carob Brown (Ridgway), with a smooth epispore (not slightly echinulate as stated by Sydow and Butler); contents granular, whence the spores present a minutely pitted appearance; diameter ranges from 9 to 14μ with a mean of 11.1μ .

On *Cymbopogon pendulus* Stapf at Banarhat, Doars, Bengal.

XV. (1) Collected by I. H. Burkill on *Cymbopogon pendulus* at Banarhat, Doars, Bengal, on 8 September 1908. *Type*.

Diameter (μ)	9	10	11	12	13	14	
Frequency (n)	14	22	87	57	16	4	= 200

Mean = 11.1μ .

The smut does not agree with any of the species of *Sphacelotheca* or *Ustilago* reported on other species of *Cymbopogon* either by Zundel (1938) or others.

XVI. AN UNDESCRIBED SMUT ON *DINEBRA RETROFLEXA* PANZER

A smut on *Dinebra arabica* Jacq. (= *D. retroflexa* Panzer) was collected by I. H. Burkill at Banarhat, Jalpaiguri District, on 24 August 1908 and sent to the *Herbarium Cryptogamae Indiae Orientalis*

for identification. Beyond placing it in the genus *Ustilago*, no further effort was made to establish its identity. For reasons stated below the smut is considered to be an undescribed species of *Sphacelotheca*, and the name *S. Dinebrae* is proposed for it.

***Sphacelotheca Dinebrae* Mundkur spec.nov.**

Soris omnia ovaria spiculae cujusque infectae omnino destruentibus (neque tamen ea omnium spicularum totae inflorescentiae), ovoideis, nigris, viridi-tinctis, 3-4 mm., longis, membrana cornea, persistenti, e cellulis olivascentibus agglutinatiss constituta cinctis; aliis (maturis) columella indistincta et sporis pulverulentis praeditis; aliis (immaturis) columellam manifestam et sporas centripetali-productas exhibentibus. Sporis subglobosis vel oblongo-angulatis, Raw Umber (Ridgway), epispora nonnihil lata, crasse contigueque verrucosa praeditis, 5-7 (in medio 5.8) μ diam.

Hab. In ovariiis *Dinebrae retroflexae* Panzer, Banarhat, Jalpaiguri District, Bengal, Indiae or. (I. H. Burkill, Aug. 1908.)

Ovaricolous, sori entirely destroying the ovary, all the ovaries in a spikelet being destroyed but not all the spikes in the inflorescence being smutted. Sori, oval, black with a slight greenish tinge, 3-4 mm. long, with a horny, persistent covering consisting of an olivaceous green, agglutinated layer of cells. In mature sori the columella is not clearly distinguishable and the spores are pulverulent. In immature sori a prominent columella is present and the spores are formed in a centripetal manner. Spores subglobose to oblong-angular with a coarsely and closely verrucose rather thick epispora; diameter ranges from 5 to 7 μ with a mean of 5.8 μ .

On *Dinebra retroflexa* Panzer at Banarhat, Bengal. Collected by I. H. Burkill on 24 August 1908. Type specimens deposited in *Herbarium Cryptogamae Indiae Orientalis*, New Delhi, and the Imperial Mycological Institute, Kew, England.

XVI. (1) Collected by I. H. Burkill on *Dinebra retroflexa* at Banarhat, Jalpaiguri District, Bengal, on 24 August 1908. *Type*.

Diameter (μ)	5	6	7	
Frequency (n)	27	159	14	= 200
Mean = 5.8 μ .				

W. McRae, who studied the germination of the spores at the time, has left a note stating that they germinate in water, but not freely, forming a promycelium, with transverse walls, and conidia. He suggested that the smut comes near *Ustilago Kusanoana* P. Hennings (1904). *U. Kusanoana* possesses a hard false membrane, but its spores are $6-9 \times 5-7 \mu$ and therefore much larger. It occurs only on *Eragrostis ferruginea* P.B., a grass not even closely related to *Dinebra retroflexa*.

The genus *Dinebra* belongs to the tribe Chlorideae and is closely

related to *Chloris*, *Cynodon*, *Dactyloctenium*, *Eleusine*, *Leptochloa* and *Microchloa*. The smut on *Dinebra retroflexa* stands out from those reported on the species belonging to these genera by its small and closely verruculose spores and its horny "cuticle".

XVII. *USTILAGO CORNUTA* SYD. & BUTLER

The smut on *Ophiurus corymbosus* Gaertn. f. was collected by E. J. Butler on 16 August 1903 at Surat and described as a new species by Sydow & Butler (1906). A portion of the type specimen, Butler, no. 447, is available in the *Herbarium Cryptogamae Indiae Orientalis*. The fungus was only once collected. The presence of a smoky coloured membrane and of a prominent columella, which extends above the sorus into a curved structure, undoubtedly places the smut in the genus *Sphacelotheca*.

Sphacelotheca cornuta (Syd. & Butler) Mundkur comb.nov.

Culmicolous; sori destroying the spike, but partially destroyed spikes, which Sydow and Butler speak of as rare floral infections, occasionally occur; false membrane smoky brown, consisting of hyaline, globose cells, 9–14 μ in diameter, at first present, very soon flaking away and revealing a black spore-mass that surrounds a long, simple columella. Spores mostly globose, Carob Brown (Ridgway), with a slightly thick smooth epispore but with spore contents minutely granular giving the spores a faintly pitted appearance; diameter ranges from 5 to 8 μ with a mean of 6.7 μ . Syn.: *Ustilago cornuta* Syd. & Butler.

On *Ophiurus corymbosus* Gaertn. f. at Surat. Collected by E. J. Butler on 16 August 1903.

XVII. (1) Collected by E. J. Butler on *Ophiurus corymbosus* at Surat on 16 August 1903. *Type*.

Diameter (μ)	5	6	7	8	
Frequency (n)	4	79	107	10	= 200
Mean = 6.7 μ .					

XVIII. *USTILAGO ROTTBOELLIAE* SYD. & BUTLER

This smut was collected on 28 July 1907 by E. J. Butler on *Rottboellia compressa* at Pusa and described as new by Sydow & Butler (1907). It has not been collected anywhere else, since then, in this country. According to Blatter & McCann (1935) the name of the host accepted at present is *Hemarthria compressa* (Linn. f.) R.Br. Butler's specimen no. 723, which is part of the type, is available in the *Herbarium Cryptogamae Indiae Orientalis*.

The smut has a very definite, brown, false membrane and a prominent columella, and hence is a *Sphacelotheca*.

Sphacelotheca Rottboelliae (Syd. & Butler) Mundkur comb.nov.

Culmicolous; entire inflorescence destroyed by the sorus which may be 5-7 cm. long. Sori covered by a false membrane which flakes away into globose, hyaline, smooth, sterile cells; columella simple but prominent. Spores pulverulent, deeply brown in mass, mostly ellipsoidal, subglobose to globose, and Kaiser Brown (Ridgway), with smooth epispores and granular contents hence appearing minutely granular; diameter ranges from 5 to 9 μ with a mean of 7.3 μ .

On *Hemarthria compressa* (Linn. f.) R.Br. (= *Rottboellia compressa*) at Pusa. Collected by E. J. Butler on 28 July 1907. Syn.: *Ustilago Rottboelliae* Syd. & Butler, *U. Rottboelliae* Miyake, *Cintractia densa* McAlpine, and *Sphacelotheca densa* (McAlp.) Cif.

XVIII. (1) Collected by E. J. Butler (no. 723) on *Rottboellia compressa* (= *Hemarthria compressa*) at Pusa on 28 July 1907. Type.

Diameter (μ)	5	6	7	8	9	
Frequency (n)	3	34	114	27	22	= 200
Mean = 7.3 μ .						

A smut was described on the same host by McAlpine (1910) and named *Cintractia densa*. It was transferred to *Sphacelotheca* by Ciferri (1928). As the descriptions given by McAlpine (1910) and by Zundel (1938) agreed in general with that of *S. Rottboelliae*, a fragment of the type specimen of *S. densa* was obtained from the Herbarium of the Department of Agriculture, Victoria, Australia, for comparison. The two specimens agree very well and there is little doubt that *S. Rottboelliae* and *S. densa* are the same. The former specific epithet has priority.

Spegazzini (1925), while revising the smuts attacking the species of *Stenotaphrum*, referred to the following as synonyms of *Ustilago affinis* Ellis & Everhart: *U. Stenotaphri* McAlp., *U. Stenotaphri* P. Henn., *U. Stenotaphri* Masee and *U. americana* Speg., while Ciferri (1931) suggested that *U. Rottboelliae* Syd. & Butler and *U. Rottboelliae* Miyake be also added to the synonymy. Of these, the smuts described by Hennings, Masee, and Spegazzini were on *Stenotaphrum glabrum* Trin. which was once considered to be the same as *Rottboellia compressa* Beauv. non Linn. f. The Indian *R. compressa* is the one described by the younger Linnaeus and is now known as *Hemarthria compressa* (Linn. f.) R.Br.

In a later publication (Ciferri & Herter, 1932), Ciferri corrects his previous statement as a result of an examination of the type specimens of *Ustilago affinis* and *U. Rottboelliae* Syd. & Butler, and states that they are distinct. The description given by Miyake (1913) leaves little doubt that his fungus is also *Sphacelotheca Rottboelliae* (Syd. & Butler)

Mundkur, and this it is hoped to establish with certainty after a comparison of the types. The distribution of the species would then be India, China, Australia and South Africa. *S. Rottboelliae* differs from *S. flagellata* (Syd.) Zundel in having smooth and smaller spores.

The genus *Rottboellia* is very closely allied to *Ophiurus* and *O. corymbosus* was, in fact, originally called *Rottboellia corymbosa* by the younger Linnaeus. But *Sphacelotheca cornuta* on *Ophiurus corymbosus* differs from *S. Rottboelliae* in having smaller spores, a thicker epispore and a deeper colour.

XIX. *UROCYSTIS BRASSICAE* MUNDKUR

The smut which causes galls on the roots of sarson, *Brassica campestris* L., was originally collected in the neighbourhood of Pusa in February 1920, identified at the Imperial Mycological Institute, Kew, as *Urocystis coralloides* Rostrup and again studied by me in 1938a. After comparing the spore-balls and the spores with those of the type collection of *U. coralloides* and testing the pathogenicity of the smut, the conclusion was reached that the sarson root-gall smut was a new species, which was called *Urocystis Brassicae*. A brief description is given below.

Urocystis Brassicae Mundkur

Sori in roots, forming galls which are 8–22 mm. in diameter, and which consist of spore-balls, hyaline anastomosing mycelial strands, and xylem elements of the host. Spore-mass agglutinated and black; spore-balls $25-59 \times 20-45 \mu$ with a mean of $38 \times 32 \mu$, with 1–5 fertile cells, 2 or 3 predominating. Fertile spores Liver Brown (Ridgway), $13-25 \times 9-20 \mu$, with a mean of $20 \times 16 \mu$, and surrounded by numerous, small bright-brown to hyaline, somewhat longish, sterile spores, forming a continuous layer.

In underground parts of *Brassica campestris* L. Type collected on 5 February 1935 by M. Azmatullah Khan at Pusa.

The genus *Urocystis* was founded by Rabenhorst to accommodate those smuts in which the spore-balls have an enveloping cortex of sterile cells and from one to several interior fertile cells, which alone have the power of germinating. Liro (1922) combined this genus with the earlier *Tuburcinia* of Fries, as emended by Woronin, on the assumption that both these genera have a sterile layer of cells; but in doing so he has added confusion to the taxonomy of the Ustilaginales because the members of the two genera are cytologically and morphologically dissimilar. Ciferri, Sydow, Savulescu, and Zillig follow Liro, but Dietel, Naumov, Zundel, and others do not. Should Liro's view ultimately prevail as a result of further cytological study, the name of this fungus will need re-consideration.

XX. *USTILAGO ARUNDINELLAE* BREFELD

This smut on *Arundinella* sp. was sent for determination by D. D. Cunningham to Brefeld who described it (1895) as new. A fragment of the type was kindly sent by Dr E. Ulbrich and has been carefully studied. The date and place of collection are not given and were not presumably communicated, but Brefeld stated that he received the specimen from Calcutta which may be the type locality. The species of the host does not seem to have been determined.

A smut on *Arundinella setosa* collected in the Kumaon Hills by Butler in 1907 was referred to *Ustilago Arundinellae* by Sydow & Butler (1907). A note left by Butler with this specimen in the *Herbarium Cryptogamae Indiae Orientalis* states: "I have not seen the original of this species which is not fully described by Brefeld." Comparison with the type showed at once that the smut on *Arundinella setosa* is a different species.

Examination of the type specimen shows that *Ustilago Arundinellae* is a *Sphacelotheca*; for each sorus possesses a false membrane, and a columella. It further shows that the spores are not readily released, unless the false membrane is placed in mounting medium and teased; and that the columella is slightly coiled at the apex.

Sphacelotheca Arundinellae (Brefeld) Mundkur comb.nov.

Ovaricolous; sori 2.5–3 mm. long, covered by a firm false membrane consisting of hyaline, globose, smooth, sterile cells; columella slightly coiled at the top. Spores mostly globose, some round and flattened when they have a concavity on both sides at the centre which looks like a large vacuole; mostly Vandyke Brown (Ridgway) but those that are flat, Cameo Brown; epispore markedly thick with a smooth edge, with surface minutely pitted; diameter 7–10 μ with a mean of 8.9 μ .

On *Arundinella* sp. collected only once, by D. D. Cunningham at Calcutta (?). Syn.: *Ustilago Arundinellae* Brefeld.

XX. (1) Collected on *Arundinella* sp. at Calcutta (?). Type.

Diameter (μ)	7	8	9	10	
Frequency (n)	15	21	131	33	= 200
Mean = 8.9 μ .					

XXI. *USTILAGO BURKILLI* SYD. & BUTLER

On 6 September 1906, I. H. Burkill collected at Gauripur, Mymensingh District, a smut on *Aneilema nudiflorum* R.Br., a Commelinaceous herb; it affected the ovaries, producing sori with a rusty, greyish, membrane. Sydow & Butler (1912) described it as a new

species, and a part of the type (no. 1421) is available in the *Herbarium Cryptogamae Indiae Orientalis*. The smut was collected once only and the material is very scanty. It has not been possible to demonstrate the nature of the enclosing membrane nor the presence of a columella. The description given by Sydow and Butler is not completely correct, for they state that the epispore surface is smooth, while I have found it to be minutely pitted, so that the margin presents a closely serrate appearance. Furthermore, the measurements, taken from only twelve spores, hardly present a true picture of the range.

***Ustilago Burkilli* Syd. & Butler**

Ovaricolous; entirely destroying the ovaries. Sori 4–6 mm. long, rusty in appearance and covered by a greyish membrane; the presence of a columella not made out with certainty. Spores globose, subglobose to angularly round, usually Lemon Yellow (Ridgway) but more mature spores Aniline Yellow; contents granular, surface minutely pitted giving the edges a serrate appearance; diameter ranges from 9 to 15 μ with a mean of 12.4 μ .

In ovaries of *Aneilema nudiflorum* R.Br. at Gauripur, Mymensingh District, and collected by I. H. Burkill on 6 September 1906.

XXI. (1) Collected by I. H. Burkill on *Aneilema nudiflorum* at Gauripur, Mymensingh District, on 6 September 1906. *Type*.

Diameter (μ)	9	10	11	12	13	14	15	
Frequency (n)	4	11	28	66	41	45	5	=200
Mean = 12.4 μ .								

The name of the host is incorrectly given in Saccardo (*Sylloge Fungorum*, xxiii, 607) as "*Asseilema nudiflorum*". Outside India, this smut has been reported by Sydow & Petrak (1928) in the Philippine Islands, in the inflorescence of *Aneilema malabaricum* (L.) Merr.

XXII. AN UNDESCRIBED SMUT ON *CHRYSOPOGON COERULEUS*

On 19 September 1903 C. A. Barber collected at Bilikere, Mysore, a perennial grass which was severely affected by a smut. The host, which has only recently been identified, is *Chrysopogon coeruleus* (Steud.) Watson, a name considered by some agrostologists to be a synonym of *C. montanus* Trin. An examination of the smut showed that it belonged to *Sorosporium*, of which no species have so far been reported on any member of the genus *Chrysopogon*. It does not agree with the descriptions of any of the thirty odd species of *Sorosporium* reported on *Andropogon* and other genera allied to *Chrysopogon*, and there is little doubt that it is new. The name *Sorosporium Azmatii* is proposed for it, after Mr Azmat Ullah Khan of the *Herbarium Cryptogamae Indiae Orientalis*, New Delhi.

***Sorosporium Azmatii* Mundkur spec.nov.**

Soris culmicolis, totam inflorescentiam destruentibus, modice contortis, 2-4 cm., longis, primo vagina perfecte obductis, deinde apicem columellae oculis subjicientibus. Membrana sterili primo griseo-brunnea, postea in cellulas oblongo-ovoideas vel globosas, hyalinas, diverse magnas secedenti. Massa sporarum nigra, granulari, columellam prominentem sed simplicem circumstanti. Glomerulis 50-135 μ diam., irregulariter ovoideis, opacis, e sporis 15 ad 50 compositis, firmis, sed sub pressione levi secendentibus. Sporis globosis vel subglobosis, "Liver Brown" (Ridgway), epispora crassa praeditis, aliis externis abundante verrucis circumdatis, aliis internis pallidioribus vel hyalinis et laevibus, 7-11 (in medio 9.0) μ diam.

Hab. In culmis *Chrysopogonis coerulei* (Steud.) Watson, Bilikere, Indiae or. (C. A. Barber; Sept. 1903 (Typus); id. Sept. 1904.)

Culmicolous, entirely destroying the inflorescence which is turned into a slightly coiled solitary sorus, 2-4 cm. long, effectively concealed by the leaf sheath, the tip of the columella alone later becoming uncovered; at first clothed with a greyish brown false membrane which later flakes away into oblong-oval to globose, hyaline, sterile cells of variable size, revealing a black, granular spore-mass, surrounding a well-developed but simple columella. Spore-balls 50-135 μ in diameter, irregularly elliptical, opaque, breaking up under slight pressure, otherwise semi-permanent and composed of 15-50 spores. Spores globose to subglobose, Liver Brown (Ridgway), with a diameter range of 7-11 μ and a mean of 9.0 μ ; epispora thick, outer spores abundantly verruculate all round, inner spores less brown to hyaline and smooth.

On *Chrysopogon coeruleus* (Steud.) Watson at Bilikere; collected by C. A. Barber on 19 September 1903. Type specimens deposited in the *Herbarium Cryptogamae Indiae Orientalis*, New Delhi, and the Imperial Mycological Institute, Kew.

XXII. (1) Collected by C. A. Barber on *Chrysopogon coeruleus* at Bilikere, Mysore, on 19 September 1903 and 7 September 1904. *Type*.

Diameter (μ)	7	8	9	10	11	
Frequency (n)	9	16	132	36	7	=200
Mean = 9.0 μ .						

Clinton (1904) reported an ovaricolous smut, *Sphacelotheca Chrysopogonis* Clinton, with obscurely verrucose spores, chiefly 8-11 μ in diameter, on *Chrysopogon nutans* from Mexico, and a foliicolous smut, *Tolyposporella Chrysopogonis* Atkinson, with smooth spores, chiefly 9-12 μ in diameter, from Alabama. Both these smuts are clearly distinct from *Sphacelotheca Azmatii*.

XXIII. *UROCYSTIS SOROSPORIOIDES* KOERNICKE

The smut on a species of *Delphinium* (either *D. denudatum* Wall. or *D. vestitum* Wall.) was collected at Simla in July 1935, and this record has already been published by me (1938c). A brief description of the smut is given below.

Urocystis sorosporioides Koernicke

Foliicolous and culmicolous; sori erumpent, elliptical to circular. Spore-mass black, pulverulent. Spore-balls ellipsoidal-globose or oblong, compact, opaque, $19-51 \times 24-61 \mu$ average $29 \times 38 \mu$; each spore-ball with three to seven fertile cells surrounded by a layer of light-coloured secondary sterile cells. Fertile cells spherical to hemispherical, $11-16 \mu$ in diameter with smooth, dark brown epispore. Sterile cells $8-12 \mu$ in diameter with smooth, brown to subhyaline cell wall.

On *Delphinium* spp. at Simla. Collected by B. B. Mundkur in July 1935 and by K. Bagchee at Chakratha (latter specimen not critically examined).

The fungus forms numerous pustules on both sides of the leaf and the petiole, just below the epidermis. Reasons for not placing it in the genus *Tubercinia* are already stated (see note XXI).

XXIV. A NEW SMUT ON *PENNISETUM CILIARE* LINK

Smutted plants of *Pennisetum ciliare* were collected on the Ridge at Delhi on 3 August 1938. The smut proved to be a species of *Sorosporium* and when compared with those species of Ustilaginaceae that have been reported on the genus *Pennisetum* and the closely allied *Cenchrus*, it was found to be new. The name *Sorosporium Penniseti* is proposed for it.

It should be noted that *Pennisetum ciliare* has also been called *P. cenchroides* Richt., and *Cenchrus ciliaris* Linn.

Sorosporium Penniseti Mundkur spec. nov.

Soris in ovariiis evolutis, eaque omnino destruentibus, 3-5 mm. longis, initio inter glumas occultis et membrana manifesta, albido-grisea, apice a poro magno dehiscenti cinctis. Columella conspicua, interdum furcata, inter massas sporarum atras, granulares producta. Glomerulis late ovoideis vel irregulariter globosis, pro more 35-58, raro ad 65μ , diam., funebre-castaneis, opacis, secedentibus, e sporis 20 vel pluribus compositis. Sporibus globosis vel ovoideis, "Cinnamon Brown" (Ridgway), epispora gracili, tenuissime echinulata praeditis,

7-11 (in medio 9.7) μ diam. Tubibus germinationis sporidiolis destitutis.

Hab. In ovariis *Penniseti ciliaris* Link, Delhi, Indiae or. (M. A. Khan; Aug. 1938.)

Ovaricolous. Sori entirely destroying the ovaries, 3-5 mm. long, at first concealed by the enveloping glumes, and covered by a conspicuous whitish grey membrane, dehiscing to form a large pore at the apex. Columella very prominent, sometimes forked, traversing the black, spore-masses. Spore-balls broadly ellipsoidal or irregularly globose usually 35-58 μ , a few up to 65 μ , dark brown, opaque, evanescent with 20 or more spores per spore-ball. Spores globose to ellipsoidal Cinnamon Brown (Ridgway), diameter ranging from 7 to 11 μ with a mean of 9.7 μ , minutely echinulate; epispore thin. Germination by germ tubes without the formation of sporidia.

On *Pennisetum ciliare* Link at Delhi. Collected by M. A. Khan on 3 August 1938. Type specimens deposited in *Herbarium Cryptogamae Indiae Orientalis*, New Delhi, and at Imperial Mycological Institute, Kew.

XXIV. (1) Collected at Delhi on *Pennisetum ciliare* on 3 August 1938. *Type.*

Diameter (μ)	7	8	9	10	11	
Frequency (n)	2	6	76	90	26	= 200
Mean = 9.7 μ .						

This species shows affinities with, but differs in certain essential characters from, the following which are given by Ciferri (1931 *b*) in the key to the smuts affecting species of *Pennisetum* and of the allied genus *Cenchrus*.

Tolyposporium Penicilliariae Bref., reported on *Pennisetum typhoides* (and of which the type specimen has been re-examined), differs from *Sorosporium Penniseti* in having no columella, in possessing more permanent spore-balls and smooth or slightly pitted spores of 8-13 μ diameter.

Tolyposporium senegalense Speg., reported on the same host and described by Saccardo (*Sylloge Fungorum*, xxiii, 620) and by Ciferri (1931 *a*), agrees with *T. Penicilliariae* in all except that it has larger spores with a different kind of epispore marking. As such it decidedly differs from *Sorosporium Penniseti*.

Ustilago Penniseti Rabenh., of which the type specimen has been re-examined, differs from *Sorosporium Penniseti* in possessing neither a conspicuous false membrane, nor a prominent columella, and in having spores that are pitted or punctulate, rather than echinulate as in the latter.

Sorosporium Cenchrus (Bref.) Zundel reported on *Cenchrus ciliaris* Linn. (= *Pennisetum ciliare*) differs from *Sorosporium Penniseti* in having more

permanent spore-balls and smooth spores ranging in diameter from 7 to 8 μ .

Sorosporium Syntherismae (Peck) Farlow, of which Sydow's *exsiccata* no. 348 has been examined and which has been recorded on several species of *Cenchrus*, differs from *Sorosporium Penniseti* in being culmicolous; the spores at the periphery of its spore-balls are verruculose, those within smooth, and their diameter varies from 9 to 13 μ .

There is little doubt therefore that *Sorosporium Penniseti* is a previously undescribed species on *Pennisetum ciliare*.

XXV. A NEW SMUT ON *POLYTOCA BARBATA* STAPF

Smutted racemes of a grass were collected by G. S. Kulkarni at Gosenhatti, Belgaum District, on 2 September 1916. The host was identified as *Coix Lachrymā-Jobi* L. and the smut as *Ustilago Coicis* Brefeld. A comparison of the smut with Brefeld's type (see Note XIV) showed that the identification was incorrect, and the grass itself is now identified as *Polytoca barbata* Stapf (= *Coix barbata* Roxb., = *Chionachne Koenigii* (Spr.) Thw.). The smut does not agree with any of those reported on the genera allied to *Polytoca*, and none has been recorded either on this genus or on *Chionachne*. There is little doubt then that it is undescribed and the name *Ustilago Polytocae* is proposed for it. It was collected again on the same host by Mr Kulkarni on 30 October 1916 on the Charodi Farm.

Ustilago Polytocae Mundkur spec. nov.

Soris in ovariis nonnullis, aliquanto amplioribus racemi evolutis, tegumento hospitali coriaceo, lugubre brunneo cinctis, apice dehiscentibus. Sporis pulverulentis, irregulariter subglobosis et aliquanto angulatis, lugubre "Hessian Brown" (Ridgway), epispora crassa, aculeis gracilibus, brevibus et obtusis ornata, sed foveolis carenti, praeditis, 9-15 (in medio 12.4) μ .

Hab. In ovariis *Polytoca barbata* Stapf; Gosenhatti, Belgaum District, Indiae or. (G. S. Kulkarni; Sept. 1916.)

Ovaricolous but not all ovaries in a raceme attacked; male flowers not smutted; ovaries slightly enlarged, sori deep brown, covered by a thick leathery membrane of host tissue. Spores released by a tear at the apex of the membrane, pulverulent, deep Hessian Brown (Ridgway), irregularly subglobose and slightly angled with a thick epispora; with fine, blunt, short spines, but no pits; diameter from 9 to 15 μ with a mean of 12.4 μ .

On *Polytoca barbata* Stapf at Gosenhatti, Belgaum District. Collected by G. S. Kulkarni on 2 September 1916. Type specimens in the *Herbarium Cryptogamae Indiae Orientalis*, New Delhi, and the Imperial Mycological Institute, Kew.

XXV. (1) Collected by G. S. Kulkarni on *Polytoca barbata* Stapf at Gosenhatti, Belgaum District, on 2 September 1916. Type.

Diameter (μ)	9	10	11	12	13	14	15	
Frequency (n)	3	3	39	71	57	28	5	=206
Mean = 12.4 μ .								

XXV. (2) Collected by G. S. Kulkarni on *Polytoca barbata* Stapf on the Charodi Farm on 30 October 1916.

Diameter (μ)	10	11	12	13	14	15	
Frequency (n)	3	23	65	84	18	7	=200
Mean = 12.6 μ .							

This smut differs from *Ustilago Coicis* Brefeld in possessing spores that are distinctly larger and have a thicker deeper brown epispore, which displays no prominent pits.

SUMMARY

This paper records the results of a critical re-investigation of forty-four collections of Indian smuts in the *Herbarium Cryptogamae Indiae Orientalis* of the Imperial Agricultural Research Institute at New Delhi. Most of the hosts have been re-identified and the smuts themselves compared with fragments of the type collections. The forty-four collections have grouped themselves into twenty-five species, of which seven are proposed as new and five appear as new combinations. The type collection of the exotic *Ustilago spermoidea* Berk., received from Peradeniya, Ceylon, was found to belong to the genus *Sphacelotheca*, and the species is therefore transferred to that genus. Further work with the rest of the collections is in progress.

[Since I wrote in the introductory remarks that 109 smuts had been recorded for India, one more smut, *Cintractia disciformis* Liro, has come under my notice. Though the smut was collected by Duthie in 1884, it was described only in 1935 by Liro as a new species in the Finnish Journal, *Annales Botanici Societatis Zoologicae-Botanicae Fennicae Vanamo*, VI, 6, 1935.]

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PROCEEDINGS

Meeting held at University College, London, 26 November 1938

The President, Miss K. SAMPSON, in the Chair

Dr W. R. S. WORTLEY (School of Agriculture, Cambridge). The effect of lithium on mildew and wheat. A summary.

The observation by Spinks (*J. Agric. Sci.* v, 231-47, 1913) that the addition of small quantities of lithium to the soil resulted in a decrease in the susceptibility of wheat to *Erysiphe graminis* DC. has been confirmed in pot-culture experiments with seedlings of two varieties of wheat. 50 c.c. of a 1% solution or 20 c.c. of a 2.5% solution of the nitrate, chloride or sulphate of lithium applied to the soil in a four-inch pot brought about a high degree of resistance to *E. graminis* without harming the seedlings.

A decrease of 40% in the number of lettuce seedlings attacked by *Marssonina Panattoniana* Berl. has been obtained in the same way, but the treatment brought about only a slight decrease in the susceptibility of wheat to *Puccinia triticea* Erikss., and this result was obtained only with amounts of lithium which were somewhat toxic to the seedlings.

A cytological investigation of the resistance of wheat to powdery mildew brought about by lithium has been carried out on epidermal strips taken from inoculated leaves and stained with cotton blue. The fungus shows no apparent toxic effects in attempting to penetrate the epidermis of the resistant plant. The cuticle is penetrated normally, but usually no haustorium becomes established. From a comparison of the staining reactions in the epidermal wall surrounding the point of penetration in the resistant and susceptible plants, it is deduced that the resistance brought about by lithium is probably due either to an alteration in the subcuticular part of the epidermal cell-wall or to the inactivation of an enzyme of the fungus.

Dr E. M. FRAENKEL (L.C.C. Southern Group Laboratory, Park Hospital, London, S.E. 13). Allergic diseases and moulds.

Among the air-borne causes of allergy in cases of asthma or rhinitis, moulds seem to play a considerable role, especially in countries with wet climates such as England or certain parts of the United States. This has been established by skin tests with mould extracts in hypersensitive patients. The skin tests showed much more frequent positive reactions than those from patients in continental countries.

A film illustrating such allergen extracts, the skin reactions obtained with them, and the method of protecting the patient from the inhalation of such air-borne inhalations by filter apparatus, was shown.

The question of moulds as the cause of allergic conditions has been studied, and attempts have been made to obtain cultures of the moulds from the patient's

own environment, and to use these moulds for the diagnosis and treatment of allergic conditions. With the help of Miss F. L. Stephens a number of moulds have been identified. Some of the moulds which were found in the patient's environment—e.g. house dust, pillow feathers, or mattress stuffings—were also found in the patient's excretions. In asthmatic patients, the sputum; in cases of rhinitis, the nasal swab; and in cases of eczema, the stool of the patient; were used for culturing the moulds.

It was assumed that moulds found in the excretion and in the environment were probably in closer contact with the patient, and therefore more likely to cause allergic changes in the patient. The number of patients examined is, however, not yet great enough to draw conclusions.

Dr RALPH EMERSON (Botany School, Cambridge). Life cycles in the Blastocladales.

The phycomycetous order Blastocladales embraces three genera of aquatic fungi, *Allomyces*, *Blastocladia*, and *Blastocladiella*. The types of sexual reproduction and the life cycles which have been discovered in certain members of this group are unique in the filamentous Phycomycetes. They are of particular interest because they have an important bearing on general problems of sexuality, and alternation of generations, and on phylogeny in the fungi.

The following life histories were described:

I. *Allomyces*.

(a) *Euallomyces*—alternation of equal sporophyte and gametophyte generations; discovered in *A. javanicus* by Kniep in 1929 and 1930 and confirmed in *A. arbuscula* by Hatch in 1933, by Sörgel in 1937 and by others.

(b) *Brachyallomyces*—without alternation of generations, the sexual stage apparently entirely lacking; found by Emerson to be the regular life cycle in certain isolates, and noted by Sörgel as a departure from the usual cycle in *A. arbuscula*.

(c) *Cystogenes*—without obvious alternation of generations but differing clearly from (b) in the regular encystment of swimmers from resistant sporangia; discovered in four of his isolates and in *A. moniliformis* by Emerson in 1938.

The nuclear behaviour in all species of *Allomyces* is still in question.

II. *Blastocladia*.

The complete life cycle is not yet demonstrated in any of the species. The importance of recent work on germination of resistant sporangia by Blackwell in 1937 and on the growth of *Blastocladia* in pure culture (by Emerson) was emphasized.

III. *Blastocladiella*.

(a) Short cycle, corresponding with *Brachyallomyces*, discovered by Matthews in 1937 in *B. simplex*.

(b) Long cycle, corresponding with *Euallomyces*, discovered by Harder and Sörgel in 1938 in *Rhopalomyces variabilis*=*B. variabilis*.

The similarities and probable relationships between members of the three genera were briefly discussed.

Miss P. E. THOMAS (University College, Cardiff). Studies of species of *Monoblepharis* occurring in South Wales.

An investigation of the species of *Monoblepharis* occurring in the vicinity of Cardiff was begun in 1935, and by February 1936 all six of the fully known species of the genus had been found, growing together in one locality. Subsequent collections from this and other localities in the same area have yielded these six species in great abundance.

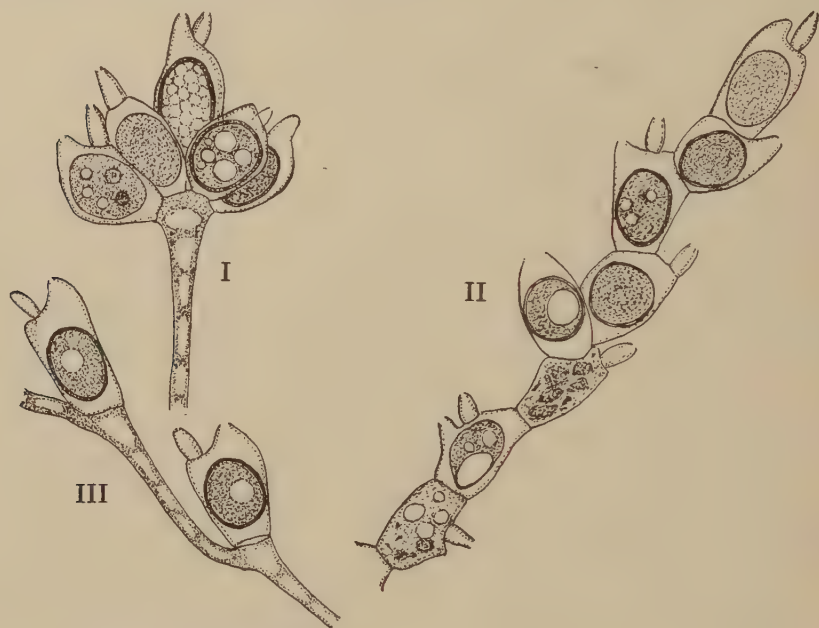


Fig. I. Fruiting tip of *Monoblepharis fasciculata* showing fasciculate arrangement of sex organs. $\times 264$

Fig. II. *Monoblepharis insignis* showing characteristic basipetal growth. $\times 264$

Fig. III. *Monoblepharis insignis* showing linear arrangement of sex organs. $\times 264$

Although waterlogged, uncorticated twigs of the birch and ash were previously suggested as the most favourable substrata, the fungus was found to grow upon twigs from a great variety of trees, the most favourable among these being oak. It seems that the consistency of the wood is of greater importance than its species.

Monoblepharis has been found to show profuse visible growths in the field only during cold weather, and although when cultivated in the laboratory it will grow at room temperatures, a period of artificially produced cold conditions favours the production of active growth.

The taxonomy of the following six species was described: *Monoblepharis sphaerica*, *M. macrandra*, *M. polymorpha*, *M. brachyandra*, *M. fasciculata*, and *M. insignis*. Particular attention was paid to *Monoblepharis fasciculata* and *M. insignis* because these two species have not been recorded anywhere, since they were originally described by Thaxter from North America in 1895.

MISS ENID MORGAN (University College, Cardiff). Studies on the Saprolegniaceae of Glamorgan.

During the years 1934 to 1936 an investigation was carried out on the relative occurrence of species of the Saprolegniaceae in soil samples from two areas in Glamorgan, concurrently with another study of the species occurring in the drainage water from these soils.

Samples of soil were taken each month over a period of two years from wet and dry pasture and from garden soil in two districts in the county. Twenty-eight species of the Saprolegniaceae were isolated.

Nine species of *Saprolegnia* and four species of *Isoachlya* were found; these occurred most frequently in wet pasture soil. Nine species of *Achlya* were isolated; they were more common in dry pasture soil than were the species of *Saprolegnia*.

Certain species with monoplanetic zoospores, e.g. *Pythiopsis Humphreyana* and *Thraustotheca clavata*, occurred more frequently in dry than in wet pasture soil. These results may indicate a relationship between the behaviour of the spores and the occurrence of the species. It is suggested that those species in which there is a reduced planetic phase may be more adapted to drier soils than those in which a diplanetic condition obtains.

Temperature seems to be a contributing factor in distribution, some species favouring a low average temperature, others a higher one. It was found that certain species are characteristic of the spring months, others the summer, while still others occur most commonly in the autumn and winter.

In certain species it is suggested that soil and water may form an alternate habitat. *Saprolegnia lapponica* was abundant in water in July, but reached its greatest frequency in soil in April and May.

The identification of species according to the existing standards of classification presents certain problems. It has been found that in different monospore cultures of the same species, the morphological features may exhibit considerable variation, and that characteristics of a particular culture may change radically in the course of a few months. This has led to the suggestion that, as our knowledge of the group increases, it may become necessary to employ physiological as well as morphological characters for taxonomic purposes.

